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CONTENTS

buffers for controllmg pH m the blood   So sensitive are they that the interven-
tion mcidental to a given test procedure may actually produce results m certain
effectors exactly the opposite of the expected peripheral change   Under 7 we
shall consider the mechanisms of maintaining "balance" or *dynamic equilibrium*

The criteria by which we may determine the sympathetic (orthosympathetic)
as opposed to the parasympathetic character of a given mechanism are varied
and not always consistent with one another   The following will be tentatively
accepted as indicators of sympathetic (orthosympathetic) as opposed to para-
sympathetic activity pending specific exceptions which may later be referred
to   1, thoraco-lumbar as opposed to cranio-sacral innervation, 2, postganglionic
as opposed to preganglionic peripheral innervation, and 3, adrenergic as opposed
to cholinergic humoral transmission of impulses to the effector   The possible
rôles of the ions potassium vs calcium, catabolic vs anabolic metabolism, dehy-
dration vs hydration, etc , may be given weight m specific instances

I *Tests based on autonomic mechanisms having a single innervation*   A *The
nictitating membrane*   The most definite indications of normal autonomic func-
tion are perhaps obtained m the case of those effectors having an innervation
from only one of the opposed branches of the autonomic system   The autonomic
innervation of the nictitating membrane of the cat, as shown by Rosenblueth and
Bard (1932) is purely sympathetic[1] and, barring certain antagonistic effects from
the skeletal external rectus muscle, eliminated by curare or deep anesthesia,
it provides an ideal sympathetic indicator   It is pharmacologically adrenergic,
being maximally sensitive to excitatory (E) sympathin (Rosenblueth and Can-
non, 1932)   That the reaction to adrenalin may be enhanced by eserin and
decreased by atropine (Rosenblueth, 1932, Secker, 1937) and that the N M
may react to large doses of acetylcholine m the animal sensitized by denervation
(Morrison and Acheson, 1938) does not seriously detract from its usefulness as
a sympathetic indicator   It has been utilized as such
an indicator of sympathetic activity, among others, by Acheson, Rosenblueth
and Partington (1936), Bargeton (1938), Rosenblueth and Schwartz (1935),
Brooks (1933), Brown (1934), Gellhorn and Darrow (1939)

As a sympathetic indicator the N M may provide an index not only of sym-
pathetic excitation, but also of a decrease or inhibition of sympathetic tone
Such effects from stimulation of the viscera (bladder and rectum) have been
reported by Watkins (1938)   Gellhorn and Darrow (unpublished) have ob-
served the effect following traction on the intestines   Rosenblueth and Schwartz
(1935) show relaxation of the N M following vagal (depressor nerve?) stimula-
tion   Relaxation of the N M or decrease of response m the presence of a rise
m the blood pressure as reported by these latter authors and by Gellhorn,
Darrow and Yesinick (1940) ͡   exaggeration of N M response reported
by Rosenblueth and Schv̇   ͡   carotid sinus denervation
does not detract from t'   ͕dicator of sympathetic
function   Rather, such   the application of this
mechanism as an indi   the moderator nerves

[1] Bucy (1927) rȧ̤   in the rabbit

ands Another group of mechanisms having a single innervation
ed as indicators of intact sympathetic nerve supply are the sweat
ons of the postganglionic nerves to the periphery produce local
the skin which may be registered as an extreme rise in the electrical
chter 1927) with no return of spontaneous activity (Tower and
After preganglionic section in the cat the rise in resistance was
r postganglionic section, restoration of function being registered
28 days (Tower and Richter, 1931, see also Hinsey Phillips and

t of sweating is provided by applying to the skin bits of filter paper
aking in a solution of cobalt chloride which changes from red to
y (Wetherell, 1905) Graded series of color changes have been
he writer by adding as hygroscopic agent varying percentages of
he cobalt chloride solution For photographic mapping of sweat
a neurologic cases the method of Minor (1928) has had wide and
plication (Guttman, 1931 List and Peet 1938–1939 Richter and
42 Hyndman and Wolkin 1941a, b) A solution of iodine and
prayed over the patient, after which he is dusted with fine starch
turns blue-black in the presence of moisture from the sweat glands
Woodruff (1941–42) have correlated the iodine-starch with the
stance methods of mapping, demonstrating their correspondence
nd the concomitant (Darrow, 1927 1932, 1934) electrical changes
pecially of the palms of the hands is not only dependent upon an
thetic supply (Richter, 1927) but is extremely susceptible to alter
ng the level of activity of the central nervous system such as
leep (Richter, 1926), and attention or alertness (Darrow, review
it must be conceded that from sleep to alert attention sympa
altered more or less in parallel with the activity manifested by the
it may be questioned whether measurements of palmar
de an uncomplicated measure of sympathetic activity In the
cat glands themselves are dependent upon cholinergic transmis-
tor (Dale and Feldberg, 1934) and conditions altering such cho-
, whether by the amount of available acetycholine the concen
inesterase, or the presence of adrenalin (Billigheimer, 1920,
rono, 1922, Darrow and Gellhorn, 1939, Darrow review, 1937)
secretory activity and the magnitude of response to stimulation
nergic limitation of sweating at the effector should be taken
(see sec V) when sweat measurements are the basis for de-
the activity in the autonomic nervous system
reflex palmar sweating in response to peripheral stimuli is in
uned by centers located in the forebrain (Langworthy and
ing and Lu, 1930, Schwartz 1936) and case of elicitation of the
dent to a very great extent upon the state of the higher levels
pointed out by others and as we have had repeated occasion
n Gellhorn and Darrow, 1941) light anesthesia, too moderate

CONTENTS

PHYSIOLOGICAL AND CLINICAL TESTS OF AUTONOMIC FUNCTION AND AUTONOMIC BALANCE

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To attain significance a test of autonomic function must circumvent the mutually antagonistic action of the two branches of the autonomic nervous system so that it may be clear whether an observed peripheral event is due to increase of activity in one branch of the autonomic system or to decrease of activity in the other   There must be no question, for example, whether an observed pupillary dilatation is due to sympathetic excitation or to inhibition of the para sympathetically determined irido-constrictor tone   The problem is literally to determine the weight on either side of a "balance" when that on neither side is known   The mere knowledge that the balance has been upset by a given condition, as afforded by many so-called tests of autonomic function, may be physiologically or clinically of little value except as indication that something has been disturbed   It does not necessarily define the foregoing events in the neural and neurohumoral systems, and in consequence may even be misleading in determining proper corrective procedures   Furthermore, peripheral autonomic events which now may bear one relation and now another to initiating processes in the nervous system need have no consistent relation to those manifestations of nervous system function known as "behavior "   This may explain the sterility which, with few exceptions, has beset attempts to correlate measurements of peripheral autonomic changes with human "behavior "

Circumvention of the difficulties in the pathway to interpretation of peripherally observed autonomic effects in terms of events in the neurohumoral system has been attempted in several ways 1, by recording changes in those exceptional mechanisms having only a single autonomic innervation, e g , in the nictitating membranes, 2, by elimination of one of the opposing dual innervations surgically, e.g , by cutting the sympathetic supply to the pupils, 3, by assaying *in vitro* or *in vivo* the output of the respective neurohumoral mediators, adrenalin or sympathin, and acetylcholine 4, by blocking one of the opposed neurohumoral mechanisms pharmacologically, e g , by administration of atropine or ergotoxin, 5, by deriving the activity of the autonomic system from the respective effects on differentially sensitive autonomic effectors, 6, by recording the electric activity of the respective autonomic nerves   The main methods of autonomic measurement will be considered in the above order

The interpretation of findings so obtained is further beset by difficulties imposed by homeostasis, since the rôle of the autonomic system is not merely to produce adaptive changes, but to maintain the internal milieu   Medullary, hypothalamic, hypophyseal, aortic and carotid sinus *feed back* "buffer" or "moderator" mechanisms are as important for neurohumoral control as are the

B *Sweat glands*   Another group of mechanisms having a single innervation and widely used as indicators of intact sympathetic nerve supply are the sweat glands   Lesions of the postganglionic nerves to the periphery produce local anhydrosis of the skin which may be registered as an extreme rise in the electrical resistance (Richter, 1927) with no return of spontaneous activity (Tower and Richter, 1932)   After preganglionic section in the cat the rise in resistance was less than after postganglionic section, restoration of function being registered electrically in 28 days (Tower and Richter, 1931, see also Hinsey, Phillips and Hare, 1939)

Another test of sweating is provided by applying to the skin bits of filter paper dried after soaking in a solution of cobalt chloride which changes from red to blue when dry (Wetherell, 1905)   Graded series of color changes have been obtained by the writer by adding as hygroscopic agent varying percentages of glycerine to the cobalt chloride solution   For photographic mapping of sweat distribution in neurologic cases the method of Minor (1928) has had wide and successful application (Guttman, 1931 List and Peet, 1938–1939 Richter and Woodruff, 1942, Hyndman and Wolkin, 1941a b)   A solution of iodine and castor oil is sprayed over the patient, after which he is dusted with fine starch powder which turns blue-black in the presence of moisture from the sweat glands   Richter and Woodruff (1941–42) have correlated the iodine starch with the electrical resistance methods of mapping, demonstrating their correspondence

Sweating and the concomitant (Darrow 1927, 1932, 1934) electrical changes of the skin, especially of the palms of the hands, is not only dependent upon an intact sympathetic supply (Richter, 1927) but is extremely susceptible to alterations affecting the level of activity of the central nervous system such as narcosis and sleep (Richter, 1926), and attention or alertness (Darrow, review 1936)   While it must be conceded that from sleep to alert attention sympathetic tone is altered more or less in parallel with the activity manifested by the sweat glands, it may still be questioned whether measurements of palmar sweating provide an uncomplicated measure of sympathetic activity   In the first place the sweat glands themselves are dependent upon cholinergic transmission at the effector (Dale and Feldberg, 1934) and conditions altering such cholinergic activity, whether by the amount of available acetylcholine, the concentration of cholinesterase, or the presence of adrenalin (Billigheimer, 1920, Langley and Uyeno, 1922, Darrow and Gellhorn, 1939, Darrow review, 1937) limit the level of secretory activity and the magnitude of response to stimulation   The apparent adrenergic limitation of sweating at the effector should be taken into consideration (see sec V) when sweat measurements are the basis for deductions regarding the activity in the autonomic nervous system

Furthermore, reflex palmar sweating in response to peripheral stimuli is in great part determined by centers located in the forebrain (Langworthy and Richter, 1930, Wang and Lu, 1930, Schwartz, 1936) and ease of elicitation of the response is dependent to a very great extent upon the state of the higher levels of the brain   As pointed out by others and as we have had repeated occasion to observe (Carlson, Gellhorn and Darrow, 1941) light anesthesia, too moderate

to interfere with sympathetic activity as indicated by nictitating membranes or other sympathetic mechanisms, decreases or may eliminate reactions in the footpads of the cat   Palmar or foot pad sweating, most commonly measured as the "galvanic skin reflex," is not, despite its single sympathetic innervation, an uncomplicated indicator of sympathetic function

C *Adrenal medulla*   That the adrenal medulla is another example of mechanisms having a purely sympathetic innervation (Elliott, 1912, 1913, Hollinshead, 1936, McFarland and Davenport, 1941) offers better justification for the utilization of circulating adrenalin as an index of sympathetic activity than the fact that it is a "sympathomimetic hormone," for whereas the production of adrenalin is by sympathetic excitation, the action of adrenalin is both excitatory and inhibitory   Aside from the fact 1, that the peripheral transmission of impulses to the adrenal glands is cholinergic and determined by conditions affecting cholinergic activity (Feldberg and Tsudjemura, 1934), and the fact 2, to be considered in more detail later, that the action of inhibitory (I) adrenalin is the inhibition of parasympathetic or cholinergic mechanisms rather than an effect on sympathetic ones, and the fact 3, that adrenalin by sensitizing the moderator nerves (Heymans, 1929) produces a degree of reflex inhibition of general sympathetic activity, circulating adrenalin (plus undifferentiated sympathin) may be considered an index of general sympathetic function   The assay of humoral mediators is considered in section III

Other mechanisms alleged to have but a single autonomic innervation such as the pilomotors, the spleen and the uterus have been variously used as autonomic indicators   Those subject to adrenergic inhibition such as the uterus are discussed in section III

II *Tests based on the elimination of the nerve supply from one of the opposing branches of the autonomic innervation*   This method has had wide application in the delimitation of responses from dually innervated mechanisms, as witness the use of vagotomy in the attempt to reduce the parasympathetic factors in circulatory changes   The method should be confined to acute experiments in order to exclude the possibility of sensitization of the humoral mechanisms whose neural counterpart it is desired to eliminate   Although maximum sensitization occurs only after nerve degeneration, even within the period of an acute experiment there appears the possibility of a degree of sensitization as suggested by the observations of Darrow and Gellhorn (1939) in the case of the pupil

A *The pupil*   One of the most useful applications of this method of control is that of cutting the cervical sympathetic supply to the pupil on one side to permit study of reactions determined by a purely parasympathetic innervation The difference between the reactions of the parasympathetically innervated pupil and those of the dually innervated structure of the opposite side gives indication also of the concomitant sympathetic activity   In corresponding manner the parasympathetic supply from the third nerve has been severed (Anderson, 1904, Ury and Gellhorn, 1939) to provide a pupil having only a sympathetic innervation   This method of experimental control provides striking experimental confirmation of the fact that it is the inhibition of parasympathetic tone rather than sympathetic excitation which is the predominating factor in

the dilatation of the iris of the cat and rabbit following painful stimuli   The sympathetic supply unquestionably also contributes to the pupillary response after emotional stimuli affecting the hypothalamus (Wang, Lu and Lau, 1932, Carlson, Gellhorn and Darrow, 1941, Hodes and Magoun, 1941) and perhaps also when the inhibitory effects of adrenalin have been eliminated (Darrow and Gellhorn, 1939)   The neural mechanisms of the response have been worked out by Karplus and Kreidl (1911), Lieben and Kahn (1930), Bain, Irving and McSwiney (1935), Harper, McSwiney and Suffolk (1935), Harper and Mc Swiney (1937), McSwiney and Suffolk (1938)   The evidence that inhibition of parasympathetic tone plays an important, if not a predominating rôle (possibly different in the monkey, (Bender, 1938, Bender and Siegal, 1940) in a re sponse which has often been referred to uncritically as indicating "sympathetic" activity may well lead to a re-examination of the evidence behind similar inter pretations in the case of other dually innervated mechanisms

B *Blood pressure*   Perhaps nowhere is such a re-examination more appropriate than in the case of blood pressure   Too often it is uncritically assumed that a sympathetic-like rise in blood pressure in the intact animal is an indica tion of sympathetic activity, and that a parasympathetic like fall in pressure indicates parasympathetic activity   That the rise may actually be due to de crease of parasympathetic tone and that a fall may be the result of inhibition of sympathetic tone, as for example by action of the carotid sinus, is often dis regarded   The same workers sometimes committing this oversight may take great pains at other times to eliminate the ambiguity of blood pressure interpre tation by vagotomy, carotid sinus denervation, atropinization sympathectomy, and so on   The prevailing lack of correspondence between blood pressure changes and changes in valid sympathetic indicators such as the nictitating membranes, as shown by studies of Acheson, Rosenblueth, and Partington (1936), Rosenblueth and Schwartz (1935), and Watkins (1938) among investi gators already mentioned in this paper is proof of the ambiguous significance of blood pressure, assuming of course that the sympathetic system discharges as a whole (Cannon, 1915, 1920, Bard 1928, 1929)   Even after hypothalamic stimulation it was shown by Carlson, Gellhorn and Darrow (1941) that some of the sympathetic like effects commonly associated with rise in blood pressure may be due to inhibition of parasympathetic tone   On the other hand the fact that Bronk Pitts and Larrabee (1940) have demonstrated an inhibition of sym pathetic impulses in the nerve to the heart following increase of blood pressure in the carotid sinus, and the fact that section of the nerves to the carotid sinus and other moderator mechanisms results in a chronic hypertension of varying duration as shown by Heymans and Bouckaert (1931, 1935), Bacq, Brouha and Heymans (1932, 1934), which can be prevented by prior complete sympathec tomy (Heymans and Bouckaert, 1935, Grimson, 1940) points to carotid sinus inhibitory control of the sympathetic system as a factor in blood pressure.

Autonomic control of factors in blood pressure such as heart rate and stroke volume, venous and pulmonary return and vasoconstriction and dilatation should receive separate consideration   Two will be discussed

C *Pulse rate*   The indiscriminate use of pulse rate as an index of sympa

thetic function in the intact animal is perhaps more to be deprecated than the uncritical employment of blood pressure for that purpose   Not only is pulse rate, like blood pressure, a function of the balance or "resultant" of sympathetic and parasympathetic influences (Rosenblueth and Simeone, 1934, Brown and Eccles, 1934 a, b) but by action of the aortic, carotid sinus, and other moderator nerves upon sympathetic and vagus centers, there tends to be a compensatory slowing of the heart when there is a rise in pressure, and an acceleration with a fall   In addition there is the direct effect of pressure within the heart itself The clinical value of pulse rate as an indicator depends little or not at all upon its rôle as a sympathetic-parasympathetic indicator (Henderson, Haggard and Dolley, 1927)   Even as an index of emotional changes it is of less value than blood pressure (Armstrong, 1938), and its physiologic interpretation is questionable (Gantor, 1925, Shock and Schlatter, 1942)   Recorded along with simultaneous changes in blood pressure, however, it may provide valuable indication of compensatory activity by the moderator nerves which should be taken into account in the interpretation of an autonomic change   Pulse rate in the chronically denervated heart is of course determined by humoral mechanisms, to be considered later

D *Vasomotor activity*   This is a most important variable determining blood pressure, though obviously not the only one   The inference that there has been vasoconstriction when rise in blood pressure is the sole observation is at best a loose manner of speech   And the frequent identification of vasoconstriction with sympathetic activity and of vasodilatation with parasympathetic action is likewise often in disregard of the facts   Not only do vasomotor functions represent in many instances the resultant of a balance between opposed neurohumoral influences, a change in either one of which may alter the vasomotor tone, but the existence of both sympathetic cholinergic vasodilators (Euler and Gaddum, 1931, Bulbring and Burn, 1934, 1935, Sherif, 1935) and adrenergic vasodilators (Rosenblueth and Cannon, 1935, Wyman and Tum Suden, 1936) is always a threat to such interpretation   Sympathetic vasodilatation has long been recognized as a common characteristic of muscle (Hoskins, Gunning and Barry, 1916, Hartman and collaborators, 1928a, b, and Clark, 1934) where it obviously may serve an emergency function   It is particularly marked in the dog and the hare, "animals of the chase" not only in muscle but in other structures (Langley and Dickenson, 1890, Burn, 1938) and is demonstrable without ergotoxin or eserin   Room (1938) indicated that at least in some cases adrenergic dilatation may be confined to the capillaries and constriction to the arteries and arterioles   However, the skin and splanchnic region of most animals including man most frequently and under most conditions manifest sympathetic adrenergic vasoconstriction and cholinergic vasodilatation

Of great importance is the fact that evidence indicates the presence of sympathetic cholinergic vasodilator activity in many blood vessels where there are no demonstrable parasympathetic nerve connections   It thus becomes possible readily to explain in these structures the vasodilator effects of parasympathetic drugs and to account at the same time for the depressor effects of weak sympa-

thetic nerve stimulation and of small doses of adrenalin. It is probable that only with strong stimulation or with larger doses of adrenalin will the inhibitory action of sympathin I or inhibitory adrenalin become strong enough completely to counteract this sympathetic cholinergic vasodilator action   The depressor action of adrenalin after ergotamine will later receive a similar explanation. Another mechanism of adrenalin depressor effects is of course the adrenalin sensitization of the carotid sinuses

Tests of vasomotor function have become of very great concern to the physiologist and clinician because of their usefulness in the attack on the problems of hypertension and other neurocirculatory disorders   These are discussed in reviews by Brown (1936) and by Weiss (1939)   They are given consideration under the present heading because of the predominantly sympathetic hyperactivity apparently involved, and the use of sympathetic nerve section as a therapeutic procedure

Tests of sympathetic vasomotor function are urgently needed to determine the probable benefits of sympathectomy   The question is always whether such radical intervention would sufficiently restore normal circulation to be justified The use of nerve block (White, 1930, Scott and Morton, 1931) to determine the probable effects of nerve section appears logical   The employment of spinal anesthesia and general anesthesia (Scott and Morton, 1930) and sleep inducing barbiturates (Craig, 1938) to test the effect of temporary elimination of central sources of sympathetic activity also provide a relatively direct approach   The rationale behind those other tests which employ what appear to be active vasodilatation technics is not so obvious, inasmuch as their operation may be quite independent of an effect on the sympathetic system   The use of fever (Brown, 1926, Adson and Brown 1929, Adson, 1936), placing the hands in hot water (Landis and Gibbin, 1933), and the employment of cholinergic drugs are examples of this approach   In so far as these latter methods are satisfactory tests of the possible benefits of sympathectomy it must be inferred that they operate by effecting a central impairment or reflex inhibition of sympathetic tone, or that they work peripherally by opposing the action of inhibitory sympathin and adrenalin on the cholinergic vasodilators

A test of sympathetic reactivity which has had wide application in the diagnosis of hypertension is the cold pressor test of Hines and Brown (1932, 1933), White and Gildae (1937)   For this test, the patient rests for 30 minutes after which one hand is immersed in ice water for 2 minutes   A rise in pressure of over 22 mm is considered indicative of a tendency toward hypertension   Recovery of the pre test level should occur in the normal person within two minutes. Of special value in the diagnosis of hypertension also is the ophthalmoscopic observation of the eyegrounds (Weiss, 1939, Keith, Wagener and Kernohan 1928, Wagener, 1933, Hallum, 1936, and Hallum and Gibson, 1938)   Tests of skin temperature (Craig, Horton and Sheard, 1933), plethysmographic volume and plethysmographic blood flow are of course useful as are also photoelectric plethysmographic technics (Hertzman and Dillon, 1938, 1940) especially when combined with stimulating procedures of the types described above

III *Assay of the output of humoral mediators adrenalin or sympathin and acetylcholine*   The evidence that the transmission of nerve impulses to the effector organs depends upon the mediation of the humoral agents acetylcholine and sympathin needs no elaboration (Dale, review, 1935, Cannon and Rosenblueth, 1937, Butt, review, 1937)   The assay of the humoral mediators may be accomplished *in vitro* either by chemical tests or by strips of excised sensitized tissue, and it may be accomplished *in vivo* either in the same or in a second animal by registration of effects on denervated sensitized organs   It should perhaps be pointed out that any humorally determined autonomic response in the absence of nerve activity is, in effect, an *in situ* biological assay of humoral mediator

A *Assay of acetylcholine*   Space permits only mention of some of the organic preparations which have been employed   Quoting from Cannon and Rosenblueth (1937)

According to Gaddum (1936) the relative sensitiveness, measured in γ (0 001 mgm) per liter, of common indicators of acetylcholine, is as follows

Leech muscle (isolated and treated with eserine)	2
Rabbit auricle (isolated and treated with eserine)	4
Frog heart (Straub method)	10
Mouse intestine	10
Rabbit intestine	20
Frog's rectus abdominis (isolated and treated with eserine)	20
Cat's denervated gastrocnemius	100

Since none of these organs is strictly specific, the suggestions of Chang and Gaddum (1933) as to ways of distinguishing between their reaction to acetylcholine and to other substances become important

Other tests may be mentioned, such as the slowing of the denervated heart and the hypotensive effect (blocked by atropine) of acetylcholine on the blood pressure of the eviscerated chloralose cat

B *Excitatory and inhibitory sympathin and adrenalin*   The fact that sympathin, mediating the sympathetic effects is of two types, E and I, excitatory and inhibitory (Cannon and Rosenblueth, 1933, 1935, 1937) must be given more than passing attention   The more rarely mentioned evidence that adrenalin itself is also of two types (Bacq, 1933, 1934, 1940, Greer, Pinkston, Baxter and Brannon, 1938) one of which is primarily excitatory and the other of which is both excitatory and inhibitory is of no less importance [2]

It has been pointed out by Bacq (1934) that the two types of sympathin, E and I, correspond in their effects to those of an undifferentiated adrenalin and those of an adrenalin, "nor adrenalin," partially oxidized (Bacq, 1935) which has thus been deprived of its inhibitory action

Even more significant is the usually unmentioned fact that *the inhibitory action of either inhibitory sympathin, I, or of inhibitory adrenalin appears only in structures having a parasympathetic (cholinergic) nerve supply, or those in which, in the absence of a demonstrated parasympathetic supply, the presence of cholinergically*

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[2] For simplicity the excitatory and inhibitory effects of adrenalin will here be referred to as "E" and "I," respectively, analogous to Cannon and Bacq's designation of the different types of sympathin

activated effectors is indicated by a sensitivity to parasympathetic drugs For example, the contractions of the nerveless amnion of the fowl are inhibited by adrenalin (Langley, 1905, Bauer, 1928) but the amnion notwithstanding its absence of nerve supply is a cholinergically activated mechanism (Bauer, 1928) Even the cholinergic "pseudomotor" contractions of denervated skeletal muscle, most familiar in the Sherrington contraction, are blocked by adrenalin as shown by Gasser and Dale (1926), Dale and Gaddum (1930), Hinsey and Gasser (1930), Bulbring and Burn (1936), and Bender (1938) It should be emphasized that there is no clear evidence of an inhibitory action of adrenalin on purely *adrenergic* structures, except secondarily by way of effects of adrenalin on the cholinergic sympathetic ganglia (Marazzi, 1939) or by way of the carotid sinus and other moderator nerves (Heymans, 1929, Gellhorn, Darrow and Yesinick, 1939, Bronk, Pitts and Larrabee, 1940) Recognition that the antagonistic or inhibitory effects of inhibitory sympathin or adrenalin are functionally inhibitory of cholinergic mechanisms[3] (regardless of what the chemical, permeability, or other mechanisms of that antagonism may be) permits some simplification of the main facts of neurohumeral transmission and of pharmacologic action

The reaction of the *denervation sensitized nictitating membrane* is that of a *relatively* pure adrenergically excitable structure It is a sensitive indicator of excitatory (E) sympathin (Hampel, 1935, Liu and Rosenblueth, 1935, Simeone, 1937) The N M is little or not at all affected by inhibitory (I) sympathin or the inhibitory action of adrenalin. Extensive utilization of the chronically denervated nictitating membrane for the *in vivo* assay of excitatory sympathin and adrenalin by such workers as McGoun and Ranson (1937), Cattell and Wolff (1934), Rosenblueth and Morrison (1934), Partington (1936), Liu (1935), and Bender and Siegel (1940) bear testimony to its value

Differentiation of excitatory (E) from inhibitory (I) sympathin was early provided by the differential reaction of the *non pregnant uterus* of the cat and the *nictitating membranes* An amount of excitatory sympathin from stimulation of hepatic nerves, which was sufficient to contract the nictitating membrane, had relatively little inhibiting effect on the non pregnant uterus of the cat (Cannon and Rosenblueth, 1933), whereas inhibitory sympathin from stimulating inhibited structures of the gastrointestinal tract produced a marked relaxation of the uterus (see end of section) but relatively little contraction of the nictitating membrane

Further differentiation of excitatory from inhibitory sympathin was provided by the nictitating membrane in combination with the response of the pupil Cannon and Rosenblueth (1935) showed that an amount of excitatory (E) sympathin sufficient to produce a measured contraction of the nictitating membrane occasioned little dilatation of the pupil, although an amount of inhibitory (E plus I) sympathin sufficient to produce the same contraction of the nictitating membrane produced a large dilatation of the pupil Only after cutting the constrictor fibers of the iris could Cannon and Rosenblueth (1935) obtain appre-

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[3] Neobeles and Neuwelt (1938) have demonstrated an antagonism between pituitrin and acetylcholin

ciable pupillary dilatation with their sympathin E   They inferred from this that sympathin E must somehow stimulate the cholinergic constrictor mechanism   However, the evidence that inhibitory sympathin or inhibitory adrenalin relaxes the cholinergic constrictor mechanism of the iris, thus facilitating dilatation, as shown by Joseph (1916), Miller (1926), Poos (1927), Yonkman (1930), and Shackler, Christiansen and Schlossman (1937), offers another if not a better explanation   It is apparent that combined inhibitory and excitatory sympathin may work synergistically by relaxing the constrictor fibers while simultaneously contracting the dilator fibers to produce a greater dilatation than would be possible by excitation alone   Obviously the operation of the humoral mediators E and I adrenalin or sympathin on the pupil parallels the action of nerve stimulation where there may also be synergistic action of sympathetic excitation and inhibition of parasympathetic tone

When both sympathetic and parasympathetic nerve supplies have been removed the pupil in the eserinized animal provides a valuable indicator of the synergistic combined effects of excitatory and inhibitory adrenalin   Bender and Weinstein (1940) have employed such a denervated iris as an adrenergic indicator along with the denervated facial musculature as a cholinergic indicator

The gastrointestinal tract, and particularly the musculature of the large and small intestine, have provided some of the earliest and most used tests for the assay of the inhibitory action of adrenalin and sympathin   The fact is again not without significance that motility and tone are here parasympathetically or cholinergically maintained   Indeed, the intestine is itself often used as a test indicator for acetylcholine   Reflex inhibition of these structures is exemplified in the studies of King (1924), Pearcy and Van Liere (1926), Loew and Patterson (1935), and Youmans and Meek (1937)   That these inhibitory effects of nerve stimulation are neurologically sympathetic is inferred from the fact that they may be abolished by splanchnicotomy and duplicated by adrenalin   Youmans, Meek and Herrin (1938) employ both innervated and denervated Thierry fistulae in the same dog for the simultaneous testing of both the neurally and the humorally transmitted effects   That the inhibitory effects of nerve stimulation may be mediated in the gastrointestinal tract by an inhibitory sympathin indistinguishable from adrenalin is suggested by the observations of Youmans (1938), Youmans, Aumann and Haney (1939)

The rate of the denervated heart has also been used as a differential indicator for the humoral effects (Cannon and Uridil, 1921, Cannon, Lewis and Britton, 1926, Newton, Zwemer and Cannon, 1931, Rosenblueth and Phillips, 1932, Whitelaw and Snyder, 1934)   It is of course sensitive to both cholinergic and adrenergic humoral mediators, but in the absence of eserin or similar drugs the cholinergic effects may be assumed negligible, except as far as it is possible that resident acetylcholine may be subject to adrenergic inhibition   In any case excitatory and inhibitory actions, whether of adrenalin or of sympathin, would work synergistically

Coagulation time of the blood may also be used as an indicator of adrenergic effects (Cannon, 1929)

Blood sugar is another frequently used indicator in which, barring important corticoadrenal and hypophyseal effects (Soskin, 1941) changes are the resultant of opposed neuro humoral autonomic influences   The widespread identification of increased blood sugar with sympathicoadrenal function doubtless received its impetus in Cannon's early work with Britton (1925, 1927) and his emphasis on its emergency function in his book on *Bodily changes* (1929)   A degree of correspondence between changes in blood sugar and other sympathetic indicators following sympathetic stimulation is shown by Chang (1937) and Bodo and Benaglia (1938)   Such correspondence may be eliminated by sympathectomy (Bodo and Benaglia, 1938)   Although long used as an indicator of emotion in animals (Cannon and Britton, 1925, Bömer, 1930), only strong emotion is apparently effective in increasing the blood sugar of normal human subjects (Gildae, 1905), and there is general agreement that while elevation of blood sugar may be met with in depressed human subjects, the "emotions" of schizophrenic patients tend to be devoid of hyperglycemic concomitant (Bowman and Kasanin, 1929, McCowan and Quastel, 1931, Whitehorn, 1934, Gildae, Mailhouse and Morris, 1935)   The failure to obtain emotional hyperglycemia in these cases may not, as has often happened, be attributed necessarily to a defect in the sympathicoadrenal function, but may, as will appear, be equally well a consequence of an increased vagal activity and insulin secretion

Dual neuro-humoral control of blood sugar by both sympathico-adrenal and vagal insulin mechanisms was early shown in morphine hyperglycemia by Houssay and Lewis (1923)   They demonstrated that the hyperglycemia could be abolished by splanchnicotomy and that it could then be restored by cutting the vagus   They inferred the antagonistic action of the sympathetic and the vagus The rôle of the vagal cholinergic innervation of the pancreas (Clark, 1931, La Barre and Vesselovsky, 1933, Babkin, Hebb and Sergeyeva, 1939) as the factor determining the frequent absence of emotional hyperglycemia which has been the concomitant of excitation following adrenalectomy (Britton, 1925, Lumley and Nice, 1930, Harris and Ingle, 1937, McQuarrie, Ziegler Wangensteen and Dennis, 1939, and Bodo and Benaglia 1939) has apparently been overlooked until its recent demonstration as a general principle by Gellhorn, Feldman and Cortell   Under a wide range of conditions such as anoxia and convulsant drugs (1940), fever (1941), sham rage, and hypothalamic stimulation (1941), and heat and cold (1941) they demonstrate that after adreno-demedullation there is a consistent tendency for such conditions to produce a hypoglycemia which is reversed if there is subsequently a subdiaphragmatic section of the vagus to eliminate the parasympathetic innervation of the pancreas   Thus blood sugar is added to the list of indicators which must be interpreted as the resultant of a balance of opposed autonomic influences

Employing the hypophysectomized adreno-demedullated rat as an extremely sensitive indicator, Gellhorn, Feldman and Allen (1941a, b) demonstrated an abnormally high insulin content in the blood of emotionally disturbed schizophrenic patients   This probably accounts in part at least for the absence of emotional hyperglycemia in the several studies of schizophrenic patients pre-

viously cited   Related and superficially conflicting evidence will be considered
later in its relation to the problem of "autonomic balance" (see section VII)

The non-pregnant uterus of the cat, as previously noted, is another of the earlier
used indicators of adrenergic inhibitory effects   This organ is unusual in that
it is one of the few among inhibited structures having definite sympathetic inner-
vation, but, at least until recently, no established parasympathetic, pelvic nerve
innervation (Reynolds, 1939)   However, Sheehan and Labate, according to
Sheehan (1941) have now "established beyond question the presence of a para-
sympathetic (sacral) outflow "   The important consideration is that despite its
sympathetic innervation the uterus is also cholinergically activated (Reynolds,
1939)   Sherif (1935) demonstrated acetylcholine secretion by hypogastric (sym-
pathetic) stimulation in the dog

The effects of adrenalin and sympathetic stimulation is to produce relaxation
of the non-pregnant uterus of the cat as contrasted with contraction when the
animal is pregnant   This action of adrenalin in the cat is perhaps not surprising
in view of the prevalence of other adrenalinergic manifestations in this animal
Van Dyke and Gustavson (1929) and Robson and Schild (1938a, b) showed that
the change is dependent in the cat on the secretion of the corpus luteum and may
be found in pregnancy, pseudo-pregnancy, or after progesterone (see Kennard,
1937)   That the effects are mediated by an altered response to the inhibitory
factor in ordinary $(E + I)$ adrenalin and sympathin is indicated by the absence
of appreciable relaxation effect by either non-inhibitory sympathin from hepatic
nerve stimulation (Cannon and Rosenblueth, 1933) or by non-inhibitory nor-
epinephrine (Greer, Pinkston, Baxter and Brannon, 1933)   When, however,
according to these latter authors, a uterus is found which, for some reason, does
not give the usual relaxation in response to ordinary $(E + I)$ epinephrine, that
uterus will give a contraction in response to the relatively non-inhibitory nor-
epinephrine   In other words, when inhibition is not too pronounced, contrac-
tility may be demonstrated   In view of the apparent relation of adrenergic
inhibition to cholinergic function it would seem that acetylcholine may be in-
volved   Demonstration by Reynolds and Foster (1939) of acetylcholine in the
pregnant, pseudopregnant and progesterone treated uterus of the rabbit makes
this seem possible   A similar reversal of inhibitory response by physostigmine
(Agar, 1940) and by the physostigmine-like action of ergotoxine (see section IV)
also implicates acetylcholine   The limited available evidence suggests that
animals having a low level of acetylcholine or an absence of cholinergic response
to estrogens such as the nonpregnant cat (Reynolds and Foster, 1940) and the
rat (Astwood, 1940, Holden, 1939) typically show relaxation in response to
adrenalin, and that animals on the other hand which assay a higher acetylcholine
content of the uterus during estrus or pregnancy such as the rabbit (Reynolds
and Foster, 1939a, b), the dog (Sherif, 1935), and possibly by inference, the
pregnant cat (?) typically show contraction in response to adrenalin   This
suggests that adrenalin-inhibitory effects may become ineffective in the presence
of excess acetylcholine, and that in the absence of inhibition the excitatory effects
of adrenergic or cholinergic action may be clearly manifest   This is consistent

with observations on the sphincter of the iris by Joseph (1916), Poos (1927), and Yonkman (1930)   This is also the implication of the change of adrenergic relaxation to contraction following administration of physostigmine or of physostigmine-like ergotamine

There is, however, the complicating fact that during pregnancy ergotamine, while itself producing near maximal contraction of the uterus (Agar, 1940), typically reverses the adrenalin response from contraction to relaxation (Dale, 1906, Cushny, 1906, 1910)   This phenomenon has contributed no little to the confusion which has prevailed regarding the mechanism of adrenalin inhibition It is not improbable, however, that notwithstanding the fact that in general blood flow and muscular activity in the uterus vary independently (Robson and Schild, 1938), the greater susceptibility of the pregnant uterus to circulatory embarrassment is an important factor in this reaction   Vasoconstrictor reactions become more pronounced at this time (Reynolds, 1939) and any deficiency of oxygenation results in a marked increase in the tendency toward uterine con traction (Reynolds 1939) even to the point of abortion   Under such conditions adrenergic inhibition of uterine vasodilatation may decrease blood flow and occa sion contraction of the uterine musculature   On the other hand, after ergota mine, the reversal of the vasomotor response to adrenalin (Robson and Schild, 1938) may favor an improvement in circulation and result in relative muscular relaxation   The rôle of ergotamine and ergotoxine in the blocking of inhibitory adrenergic effects will be considered.

IV   *Autonomic tests involving pharmacologic blocking of one or the other branches of the autonomic system.*   This type of test has had application clinically as well as physiologically because the reversibility of the effects permits of their applica tion in human subjects

A   *Ergotamine   ergotoxine*   This drug has enjoyed a wide reputation as a "sympatholytic" drug by which sympathetic excitatory effects could be blocked pharmacologically   This has been assumed to permit the differentiation of sympathetic excitatory from other autonomic effects   That this interpretation of its action is misleading, that ergotoxin blocks primarily not sympathetic excitatory (E) but sympathetic inhibitory (I) effects (Rothlin, 1929, Thienes, 1929, Issekutz and Lenzinger, 1928), and that this action is in fact not an action on the sympathetic mechanisms but a protection of parasympathetic activity or a "physostigmine-like" action (Loewi and Navratil, 1926, Matthes, 1930, Linegar, 1939) is supported by the following facts

The primary action of ergotamine is the contraction of smooth muscle, especially of cholinergically (Sherif, 1935) activated smooth muscle such as that of the uterus (Dale, 1906, Sharp, 1911, Agar, 1940, intestine (Rothlin, 1929), excised sphincter iridis (Crouch and Thompson, 1939), stomach (Smith, 1918), and retractor penis (Dale, 1906)   In the intact animal it increases intestinal motility (Plancelles, 1925), occasions extreme miosis (Dale, 1906, Crouch and Thompson, 1939), lowers blood sugar, but not after pancreatectomy (Shpiner, 1929) and decreases blood pressure (Wright, 1930)   Frequently cases of increased blood pressure have been attributed to contraction of muscular organs   Carotid sinus

desensitization by ergotamine (Heymans, Regniers and Bouckaert, 1930, Bacq, Brouha and Heymans, 1930) may also be a factor in cases of increased blood pressure following this drug ,

Even where sympathetic excitatory effects have appeared blocked by ergotoxine, the blocking is not impossibly, as in the case of the nictitating membrane (Rosenblueth, 1932, Rosenblueth, Leese and Lambert, 1933) and the pregnant uterus (Agar, 1940), attributable to the accompanying tonic response or contracture   The sensitivity of these sympathetically innervated structures to acetylcholine has been shown in the case of the nictitating membrane by Morrison and Acheson (1938) and in the case of the uterus by Reynolds (1939)   As noted in the foregoing, even the sympathetic impulses via the hypogastric nerves to the uterus are mediated by acetylcholine in the dog according to Sherif (1935) The increased peristalsis (Planelles, 1924), increased excitability of the vagus to acetylcholine, the nausea and emesis, and the uterine contractions attending administration of ergotamine, all point to a physostigmine-like action

It is the inhibitory effects of adrenalin or sympathetic stimulation on cholinergically activated mechanisms which are blocked by ergotoxine   It is, as already noted, the ability of adrenalin or sympathetic stimulation to relax or block the spontaneous activity and tone in the intestine or the nonpregnant uterus of the cat which is prevented by ergotoxine   Even in denervated skeletal muscle adrenalin inhibition of the cholinergic pseudomotor contraction can be blocked by ergotoxine (Hinsey and Gasser, 1928)   Also in the nerve-free but acetylcholine rich human placenta (Chang and Gaddum, 1935) adrenalin constriction of the blood vessels is blocked by ergotoxine (Euler, 1938)   And even the familiar reversal of vasomotor response to adrenalin and sympathetic stimulation following ergotoxine are accounted for if we accept the evidence (Burn, review, 1939) for sympathetic cholinergic vasodilator fibers   We may assume that these cholinergic vasodilators normally are inhibited by inhibitory sympathetic action or inhibitory adrenalin, resulting thereby in a constriction which is synergic with the reaction of the adrenergic constrictors   Following ergotoxine this inhibition of the vasodilators does not take place, and there is in consequence a decreased pressor, or even a depressor effect   Bulbring and Burn (1935) and Herwick, Linegar and Koppanyi (1939) showed a similar vasomotor reversal after eserine—abolished by atropine   This again suggests that inhibitory adrenergic effects may be blocked or possibly swamped in the presence of sufficient acetylcholine   That ergotamine may likewise potentiate the depressor effects of acetylcholine and that this action may be reversed to a pressor action by atropine is shown by Linegar (1939) and by Herwick, Linegar and Koppanyi (1939)   The previously considered (section III) relation of ergotamine to the reversal of adrenergic response in the pregnant and non-pregnant cat is consistent with this point of view

That ergotamine may have considerable value as a means of testing for the presence of sympathetic inhibitory and adrenalin inhibitory effects on parasympathetic cholinergic functions appears to be indicated

B *Atropine*   The employment of atropine to block the action of cholinergic or parasympathetic mechanisms is the most familiar of pharmacologic blocking technics   Two types of measurements have been sought by this means—1, an index of the normally present parasympathetic activity derived from the changes induced when that activity is blocked, and 2, an index of sympathetic function derived from the magnitude of the total residual activity after parasympathetic opposition is eliminated

Both of these effects are to some extent obscured or damped by the possible effect of the drug upon cholinergic transmission of nerve impulses within the sympathetic ganglia and adrenals, as well as in the central nervous system, and by the possible compensatory action of the carotid sinus and other moderator nerves.

As early as 1870, Schmiedeberg demonstrated that atropine blocked the inhibitory effects of the vagus on the heart.   Escudro (1923) and Danielopolu (1926) devised tests of sympatheticotonia and parasympathecotonia based on the effects of atropine in blocking the parasympathetic control of the heart   Fentress and Solomon (1936) have applied the procedure in the study of autonomic function in psychoneurotic patients

The contrasting effects of atropine, of parasympathicomimetic and sympathicommetic drugs have been used as autonomic tests by Myerson, Loman and Dameshek (1937) and by Brohoff, Grosse and Kaldenberg (1938) in the study of the synergistic and antagonistic pharmacologic responses of human subjects   In physiological experiments the use of atropine to determine the rôle of cholinergic mechanisms in a given response is, of course, so common as to be almost routine.

V   *Equating of effects in differentially sensitive autonomic indicators*   The classic instance of derivation of autonomic activity from differential effects on peripheral autonomic indicators is Cannon and Rosenblueth's (see p 9) differentiation of sympathin E from sympathin I on the basis of the differential effects on the iris or uterus and nictitating membrane

Darrow and Gellhorn (1939) used a somewhat similar equating technic in contrasting the effects of nerve stimulation on a sympathectomized pupil having only a parasympathetic nerve supply with that of the normal pupil of the opposite side having both parasympathetic and sympathetic innervations   From the denervated pupil it was possible to determine the presence of inhibition of parasympathetic tone following stimulation, while from the difference between that and the normal pupil it was possible to infer the presence of sympathetic excitation

Darling and Darrow (1938) and Darling (1938) attempted a similar derivation from the differential effects of stimulation upon blood pressure and palmar sweating (galvanic) activity   As the method is more recently being applied (Darrow and Solomon, 1939, 1940) the rationale depends upon the assumption 1, that blood pressure change represents the difference between opposing effects of sympathico-adrenal and parasympathico-cholinergic influences   Galvanic

or sweating activity on the other hand, depends upon both sympathetic nerve conduction and upon cholinergic transmission at the effector (see p 3) These relationships are symbolized in the equations

$$BP = S - P$$

$$\text{and Galv.} = S + P$$

where S represents sympathico-adrenal activity, and P represents parasympath-ico-cholinergic activity

Given simultaneous galvanic and blood pressure responses to stimulation these simultaneous equations may be solved for either S or P Or, if desired, the data may be plotted into two-dimensional graphs or "autonomograms" The results of the method indicate simultaneous effects on both sympathetic and parasympathetic activity in the normal individual with sympathetic effects pre-dominating They indicate, further, the presence of decreased sympathetic responses and a tendency toward the inhibition of parasympathetic tone follow-ing stimulation in "resistant" hostile, inhibited, unco-operative psychotic pa-tients Evidence from the studies of Darrow and Gellhorn (1939a, b, c) that the presence of adrenalin in the circulating blood disposes toward exaggeration of responses of the type involving inhibition of parasympathetic tone, as well as a diminution of sympathetic responses, suggests that adrenalin may be a factor in determining the reaction pattern of the "resistant" psychotic patients A possible rôle of adrenalin in accounting for the condition of these patients will be considered under "Autonomic balance" (see section VII)

VI *Electrical recording of autonomic potentials* Action potentials of the autonomic effectors or of their innervating nerves provide valuable indications of autonomic activity A familiar example is the electrical recording of sweat gland activity by the use of endosomatic potentials (Tarchanoff, 1890) (See reviews of galvanic response literature, Landis and Dewick 1929, Landis, 1932, Darrow, 1936, 1937) The nictitating membranes and pilomotor muscles have been recorded electrically (Orias, 1932, Rosenblueth, Leese and Lambert, 1933, Rosenblueth, Davis and Rempel, 1936a, b, Eccles and Magladery, 1937a, b) Effects of ovulation have been registered as abdominal or vaginal action poten-tials by Burr, Hill and Allen (1935), Reboul, Davis and Friedgood (1937), and Bourdillon (1939) Effects of pharmacologic agents on uterine potentials have been studied by Bozler (1938), Morrison (1940) and Balassa and Gurd (1941) Gastrointestinal motility was studied by Bozler (1938, 1939, 1941), and paccinian corpuscle potentials of the mesentary by Gammon and Bronk (1935) Gastric impulses in the vagus are reported by Partridge and Wilson (1933) and vagal (carotid sinus?) effects by Fischer, Gantt and Lowenback (1934) Comparison of electrical responses in various types of smooth muscle such as the nictitating membranes, pilomotors, intestine, bladder, uterus and ureter by Rosenblueth, Leese and Lambert (1933), Lambert and Rosenblueth 1935, and Davis, Rosen-blueth and Rempel (1936), and Bozler (1938) indicate that the initial potentials, types I and II of Cannon and Rosenblueth (1937) are associated with excitation, whether in the case of the sympathetic nerves or in the case of the parasympathe-

tic ones   The effects of various pharmacologic agents on the magnitude of these potentials relates to effects on permeability (Rosenblueth and Cannon, 1936) Inhibitory effects are accompanied only by type III delayed and prolonged po tentials, identified by Cannon and Rosenblueth with actual contraction   In the salivary glands the possibility of differential negative as opposed to positive potentials, associated respectively with parasympathetic as opposed to sympathetic stimulation, is indicated by Bronk and Gesell (1926)   Pharmacologic effects on the potentials of the submaxillary gland were studied by Rosenblueth, Forbes and Lambert (1933)

Effects of adrenalin, atropine and other drugs in damping cholinergic transmission in sympathetic (cholinergic) ganglia, as well as the facilitating effect of moderate concentrations of parasympathetic drugs on the ganglia have been studied electrically by Marazzi (1939a, b)   Potentials from several different autonomic nerves as a means toward the solution of problems of autonomic control have been employed by Hinsey and Gasser (1930), Corbin and Hinsey (1935) and Bishop, Heinbecker and O'Leary (1934)

Especially important are the records of sympathetic action potentials and the correlation of these with cardiovascular control from the hypothalamus and carotid sinus as reported by Bronk, Ferguson, Margaria and Solandt (1936), Bronk, Lewy and Larrabee (1936), Pitts, Larrabee and Bronk (1941), and Pitts and Bronk (1942)   The rhythmic character of these potentials and their frequency at about that of the cortical potentials (notwithstanding anesthesia) is of particular interest in view of the evidence assembled by Darrow, Jost, Solomon and Mergener (1942) for an association of alpha potentials with cerebral vasoconstrictor tone

Of great interest also is the recording of potentials from the carotid sinus and the demonstration of specific functional relationships to sympathetic regulation which could have been derived only by electrical methods (Bronk, 1931, Bronk and Stella, 1932, 1935, Fischer and Löwenback, 1934, Bogue and Stella, 1934, 1935, Samaan and Stella, 1935, and Pitts, 1942)   The possible applications of such electrical recording technics (Bronk, Pitts and Larrabee, 1940) are as extensive as the ramifications of the autonomic system.

VII *Autonomic balance—dynamic equilibrium*   Autonomic dysfunction is often times referred to as "autonomic imbalance"   This may be begging the question, for obviously in a balanced system abnormal activity would be recognized only if it were sufficient to upset the balance   A dysfunction of one part of the autonomic system, balanced by a compensatory dysfunction of another part of the system might conceivably constitute a double liability, and yet not be demonstrable as an "imbalance"   The futility of treating symptoms under such conditions is obvious, and it is likely that many of the baffling problems of autonomic dysfunctions are of this type

Although the Eppinger and Hess (1909) concept of balance, of sympathicotonia, and of parasympathicotonia has served a useful purpose in directing and stimulating research, the actual clinical and physiological consequences of its application have unquestionably been disappointing   Without detracting from

the importance of the concept in the development of our thinking, it should be pointed out that present day knowledge of the physiological processes involved in maintenance of "balance" dicatates that discussion to be profitable must be concerned but little with "balance" in the abstract and concentrated rather on specific neurophysiological mechanisms of homeostatic control

Since the discovery by Hering in 1923 of the main mechanisms of autonomic regulation or maintenance of balance and the confirmation by Koch (1923) and by Heymans (1928) our knowledge of carotid sinus, aortic and abdominal vasosensory mechanisms has advanced so rapidly and the specific determinants of sympathicatonic and vagotonic effects have been sufficiently defined that reference to such terms as "autonomic balance," "sympathicotonia," and "parasympathicotonia" without physiologic qualification partakes almost of mysticism Such terms are too general and too indefinite, though on occasion it is conceded that they may be useful for purposes of classification

The carotid sinus mechanism, and also the carotid bodies, at the bifurcation of the internal and external carotid arteries, the vasosensory zone located in the arch of the aorta, and the less important, less well localized "vasotatic" receptors in the abdominal region established by Heymans (1929a, b), Heymans, Bouckaert, Farber and Hsu (1936), and Hsu and Chu (1937) supply neurosensory impulses for the reflex control of circulatory and respiratory equilibrium   The carotid sinuses exercise control by maintaining and varying the tonic inhibition over these most essential life-maintaining functions as well as over the more general emergency functions of the sympathetic system   They determine, as it were, the varying *negative bias* required to "modulate" or moderate oscillations in the circulatory system   In general, optimal conditions of oxygenation and blood pressure which favor bodily activity *activate* carotid sinus function also, and the carotid sinus in turn lowers blood pressure to that minimum which is commensurate with the maintenance of its own activity   Conditions of low oxygen, low blood pressure, or excess carbon dioxide (Bielinski and Wierzuchowski, 1939) in the carotid sinus, on the other hand, tend to reduce or *inactivate* carotid sinus inhibitory processes and release the restrained sympathetic mechanisms from tonic inhibition   At the same time tonic parasympathetic impulses from the carotid sinus are reduced   Such conditions tend, like denervation, toward hypertension, and in more extreme conditions they may occasion hyperpnea (Heymans and Bouckaert, 1930a, b, Winder, 1937a, b, 1938, Schmidt, 1932)   The terms *activation* and *inactivation* are here employed relative to the carotid sinus because of the current confusion with respect to the word "sensitization" as applied for example to Vercauteren's (1932) demonstration that in the presence of carbon dioxide the added embarrassment of a standard reduction in intrasinusal pressure will produce a larger compensatory hypertensive effect than a similar change in pressure without the carbon dioxide   Since usage has designated this effect a "sensitization" it is pointed out that in this case one agent merely potentiates the *inactivation* produced by the other (Bielinski and Wierzuchinski, 1939)   This general interpretation of carotid sinus function is offered with full consideration of the fact that in extremely low pressure, "paradoxical"

reactions with failure of respiration and blood pressure may obscure normal carotid sinus compensations   It is not assumed that these principles apply equally to the chemoreceptors of the carotid bodies, for these apparently operate by a positive rather than by an inhibitory mechanism of control

Gesell and collaborators have recently called attention to still another buffering action on ganglionic structures   They point out that acetylcholine is rapidly destroyed by cholinesterase in an alkaline medium, but that it is conserved, increasing its duration and intensity of action, in an acid medium.   This provides a direct biochemical buffering mechanism in which accumulation of acetyl choline with its facilitation of myoneural and ganglionic transmission parallels carotid sinus disinhibition of sympathetic activity

Not only do the moderator nerves normally maintain a regulatory inhibitory control over sympathetic activity, actually reducing the magnitude of vasomotor and other reactions to afferent nerve stimulation (see Rosenblueth and Schwartz, 1935, for nictitating membrane, Izquierdo, 1920, for vasomotor effects) but there is evidence likewise of a positive control by way of the parasympathetic system (Hering 1927, Heymans 1928, Bernthal and Motley, 1939)   Of effects (blocked by atropine) via the vagus on the heart there can be no question, and even increased intestinal motility and tone has been reported following carotid sinus stimulation (Tournade and Malmajac, 1929)   Evidence to the contrary (Thomas and Brooks, 1937, Grimson, 1940) that reflex carotid sinus control is completely lost following sympathectomy does not disprove the existence of normal para sympathetic regulation by the carotid sinus, for the reason that carotid sinus function is dependent both upon the presence of adequate internal pressure, and on the presence of sensitizing adrenalin, either or both of which may be eliminated by sympathectomy

Adrenalin secretion, so often identified as an emergency blood pressure raising device, apparently plays an important, and not always considered, rôle in the carotid sinus limitation and homeostatic regulation of autonomic activity   The presence of circulating adrenalin in the carotid sinus tends to sensitize that mechanism and increase inhibitory effects both on blood pressure and respiration (Heymans, 1929a, b)   This was attributed by Stella (1932) to the increase of blood pressure and the effect of adrenalin on the "centers," but Malmajac, Donnet and Desanti (1935a, b), Battencourt (1935) and Chu and Hsu (1938) showed that the presence of adrenalin actually increases the sensitivity of the carotid sinus mechanisms to the existing pressure   Thus may adrenalin occasion a depressor blood pressure reaction independently of its depressor peripheral effects previously considered   Thus adrenalin by increasing carotid sinus inhibition of the sympathetic may exercise an inhibitory control over medulliadrenal secretion   It thereby provides homeostatic limitation of its own output   In like manner may adrenalin limit the reactivity of other sympathetic functions (Darrow and Gellhorn, 1939, Gellhorn, Darrow and Yesnick, 1939)

Conversely, the action of acetylcholine in the carotid sinus may decrease inhibition of sympathetic activity as shown by Heymans, Bouckaert, Farber and Hsu (1935) resulting in hypertension   This may be considered a cholinergic inacti-

vation of the carotid sinus, since the effects are similar in direction if not in degree to those of denervation   The same apparently may be said of the physo-stigmine-like action of ergotamine in the carotid sinus, which may also result in hypertension according to Bacq, Brouha and Heymans (1932), again probably as an effect of disinhibition of the sympathetic by depression of carotid sinus reflexes, as demonstrated after ergotamine by Heymans, Regniers and Bouck-aert (1930)   The increased blood pressures observed in human subjects follow-ing ergotamine by Freeman and Carmichael (1936) are possibly so accounted for

Inhibitory control by the carotid sinus apparently extends likewise over the activities of those tonic postural mechanisms which are sometimes associated with sympathetic function   Not only will increase of intra-carotid pressure block respiration (Bouckaert and Heymans, 1930, Schmidt, 1932) but it will halt shivering (Tournade and Malmajac, 1929) decrease action potentials in skeletal muscle (Spychela, 1935) eliminate the knee jerk (Schweitzer and Wright, 1937) reduce other skeletal reflexes (Kaufman, 1938, Koch, 1932) occasion relax-ation and sleep (Koch, 1932), halt convulsions and/or induce sleep in previously narcotized animals aroused by metrazol (Gellhorn, Darrow and Yesinick, 1939) Effects such as hyperpnea, convulsions, increased muscle potentials, increased somatic reflexes and convulsions have been observed following decrease of blood pressure by the same authors   Thus conditions of autonomic equilibrium within the carotid sinus may exert a most important direct influence over the activities of the central nervous system

The most definitely identified clinical effects of carotid sinus dysfunction are probably the syncopal attacks attributed to carotid sinus hyperirritability (Weiss and Baker, 1933a, b)   Ferris, Capps and Weiss (1935, 1936) following Weiss and Baker, have subdivided such attacks into 1, cerebral, 2, cardiac, and 3, vasomotor types, depending upon the probable mechanisms of precipitation In "cerebral" attacks there is apparently a direct action of carotid sinus reflexes upon the brain or its circulation   In the other types the syncope is secondary to the embarrassment of general circulation   Convulsions in these latter cases are consequent to the syncopal attack (Freedberg and Sloan, 1937) and not directly an effect of carotid sinus hyperirritability, although Lennox, Gibbs and Gibbs demonstrate the possibility of attacks without cerebral anoxemia, by direct action of the carotid sinus on the brain   Weiss and Baker (1933) note that in idiopathic epilepsy (150 cases) carotid sinus pressure does not produce seizures Marinesco and Kraindler (1931a, b) offer further evidence that epilepsy is actu-ally associated not with carotid sinus hypersensitivity but with hyposensitivity and that it may often be a result of failure of that mechanism to protect the brain from mechanical shocks transmitted by the circulation   That a defi-ciency of the buffering mechanism is an etiologic factor in some cases of epilepsy is indeed not unlikely, but it seems to the author that electroencephalographic evidence suggests that it is the inadequate buffering of the autonomic discharges to the brain, rather than the lack of hydrodynamic control, which is probably most frequently involved

The complexity of the problem of testing and interpreting the equilibrium

which is the final product of the moderator nerves is further illustrated in certain cases of schizophrenia. Hypersensitivity and over regulation by the carotid sinus are here possibly indicated   There is a relative absence of sinus arrhythmia (Whitehorn and Richter, 1937) which, according to Regniers (1920), is a sign of carotid sinus activity   Sympathetic functions are depressed (Darrow and Solomon, 1934, 1939, 1940, Gellhorn, 1938)   Blood pressure tends to be low (Truman, Hoskins and Sleeper, 1932)   Pulse tends to be slow (Hoskins and Welch, 1932)   Postural activity may be inhibited as is evident in many of the so-called "withdrawal" symptoms   The vago-insulin secretion is increased (Gellhorn, Feldman and Allen, 1941) and emotional hypoglycemia is the rule (Bowman and Kasanin, 1929, McCowan and Quastel, 1931, Whitehorn, 1934, Gildae, Mailhouse and Morris, 1935)   Insulin desensitization with the development of an anti insulin factor may occur (Meduna and Gerty, 1932), just as insulin hypersensitivity may follow carotid sinus denervation (Casas and Hinsberg, 1932)   And a possible mechanism for such a carotid sinus sensitization in resistant hostile unco-operative patients is the secretion of adrenalin suggested by the studies of Darrow and Solomon (1940)   This is further supported by the aggravation of schizophrenic symptoms by subcutaneous adrenalin as reported by Lindeman (1935) and the decreased sensitivity to it (Kanner, 1918, and Freeman and Carmichael, 1935) which is a natural consequence of adrenalin adaptation according to Hoskins and Rowley (1915) and Rudolph (1938)   Ergotamine which desensitizes the carotid sinus has proved effective as therapy (Baber and Tietz, 1937)

We may seek, then, tests of autonomic balance in terms of specific moderator compensatory mechanisms   Symptoms of imbalance may conceivably arise *1*, from exaggeration or displacement of one type of autonomic activity such that the normal buffering mechanisms are unable to restore equilibrium, *2*, from a deficiency or insensitivity of the moderator mechanisms, *3*, displacement of activity in one part of the autonomic system and an overcompensation by the buffer mechanisms as far as other activities of the organism are concerned, and possibly *4*, from overactive or oversensitive moderator mechanisms   A tabulation of symptoms might not differentiate these conditions, yet treatment would properly differ according to the cause of the dysfunction.

Tests of autonomic balance which have had wide application are in general of two types (A) those which examine the reaction of the organism or of some of its organs to a specific autonomic stimulus, and (B) those which impose a more or less non-specific load on the organism and attempt to measure the efficiency of the equilibrium maintaining machinery under conditions of regulated stress   Such are really tests, not of static "balance" but of dynamic equilibrium   Space permits only the mention of some of the more common tests of these two types

A. *Tests of reaction to a specific autonomic stimulus*   Among such tests probably should be included the *oculo-cardiac test* (Aschner) in which pressure on the eyeballs elicits slowing of the heart, said to be marked in "vagotonic" individuals   The effects are different from those for carotid sinus pressure (Mandelsturm and Lipschutz, 1932a, b, Reguiers, 1930, Wright, 1932)   The oculocardiac effects,

probably primarily vagal, apparently affect pulse more than blood pressure Carotid sinus pressure causes both vagal stimulation and sympathetic inhibition Jacobivici and Nitzescu (1929) employ electrical stimulation of the carotid sinus producing effects similar to pressure   The cold-pressor test previously described (p 7) is probably effective primarily as a means of sympathetic stimulation That this test may be effective because it provides a harmless though moderately severe pain stimulus has probably been given less than due consideration Orthostatic tests, with or without a tilting table may be mentioned in this connection because of action on the carotid sinus   Correspondence of findings with those obtained by carotid sinus pressure appear not too good   The use of autonomic drugs by various routes should doubtless also be included in this group of tests   In the case of intravenous injections interpretation is always complicated by the fact that peripheral effects and central and carotid sinus compensatory effects may work in opposition   The compensatory effect is often the important one   Reactions to intravenous adrenalin and "wheal" and "flare" reactions to subcutaneous adrenalin may have quite different significance   Tests of reaction to carbon dioxide and to overventilation and attempts to shift the acid-base equilibrium as for example with bicarbonate or ammonium chloride deserve mention in this connection, not only because of the relation of ionic equilibrium to ganglionic and myoneural transmission, but more especially because of the activation by hypocapnia, high pH and calcium of the carotid sinus complex

It must be emphasized that *a priori* interpretations of such tests involving autonomic stimulation is hazardous   For example, the fact that pressure on the eyes produces marked vagal-like effects in certain individuals does not automatically render this a valid test of "vagotonia "   Only demonstration of the correlation of these stimulation effects with results of other valid indicators can be justification for such an interpretation

B Tests which attain significance because they impose an effective load on the organism and thus provide a means of gauging the effectiveness of various homeostatic mechanisms in handling the load are almost too non-specific to be included as autonomic tests   We should perhaps mention the Schneider (1920) test of cardiovascular efficiency, with its regulated mounting and demounting of a standard stairstep   Tests in which the ability of the organism to cope with a low oxygen pressure and acapnia also deserve mention   Apparently it makes little difference whether the subject ascends high altitudes by breathing a low percentage of oxygen from a Douglas bag, by being subjected to reduced pressure in a tank, or by boarding a plane   This adds to the facility and convenience of making such studies   A third class of tests worthy of special mention are the so-called "neurosis" producing tests which permit study of equilibrium maintaining mechanisms which may be upset in emotion

Such tests tend to go afield from the category of strictly autonomic tests Metabolic activity, brain function, electroencephalographic activity, electrocardiographic activity, biochemistry and other functions without number may be and are on occasion properly measured in pursuit of information regarding autonomic functions   The only limitation is what can be measured and what is the aspect of autonomic function under consideration

Batteries of autonomic tests have been employed for the study of autonomic function and balance with a view toward determining individual differences and to throw light on the rôle of the autonomic system in the broader clinical adaptive and psychological relations   The clinical pathological studies of Peterson and collaborators (1930–1938) provide an exploration in this field with clues to possible relationships which should be mentioned   The pharmacologic studies of Meyerson, Loman, Dameshek et al also provide a systematic approach   The correlational studies employing factorial analysis by Darling and by Wenger (1941) suggest possibilities for the application of modern mathematical technics to the solution of autonomic problems where results from batteries of autonomic tests are available   The latter method should reveal any tendency for autonomic test results to be grouped according to any principle   For example, if sympathetic and parasympathetic activities play different and determining rôles these should be derivable by such factorial analyses as indeed they were in the results of Darling   Factorial analysis, it should be pointed out, may reveal relationships which otherwise might escape attention   They cannot, however, impart autonomic significance to tests which are not themselves valid according to acceptable physiologic criteria   Such correlational procedures should nevertheless provide tests for the validity of accepted test technics and assist in the identification of still undiscovered autonomic indicators

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MUSCULAR DISORDERS ASSOCIATED WITH DEFICIENCY OF VITAMIN E

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It has become increasingly obvious from the work of the past few years that the substances designated by the term "vitamin E" play an indispensable part in the metabolism of skeletal muscle. It is the purpose of this review to assemble the evidence in support of this statement.

The first hint that the effects of vitamin E deficiency were not restricted to the reproductive sphere is to be found in the observation of Evans and Burr (1) in 1928 that the offspring of mother rats, partially deprived of vitamin E, tend to become paralyzed toward the end of the nursing period. We may quote from their description of the clinical picture "A day or two before the weaning period (21st day of life), it is frequently noted that the young in these litters have begun to have difficulty in regaining their limbs when placed on their backs. The disability increases. By the 21st day of life, about three-fourths of such young are paralyzed in part of the musculatures of the body wall and the posterior extremities. The disease increases in severity during the ensuing 4 or 5 days and by the 25th day of life, practically all animals destined to develop the disease will exhibit it, though occasional instances of a still later development have been encountered." "The suddenness of onset is remarkable. One encounters seriously affected animals known to have been normal the previous day." "Less than 20 per cent recovered, and some of these were left with residual paralysis."

"Complete protection was obtained when wheat germ oil or concentrates (non-saponifiable fraction, freed of steroids) was administered to the young." Although vitamin E had not at that time been chemically identified, the evidence pointed to a 'complicity of vitamin E in the production of this disorder.'"

Evans and Burr did not themselves investigate the underlying pathology but placed material at the disposal of Lipschütz (2) who, eight years later, published a paper describing widespread degenerative changes in the central nervous system in these rats. Curiously, the patent, grossly visible alterations of the skeletal muscles completely escaped his observation, and it was not until Olcott's (3) paper in 1938 that the paralysis of suckling rats was recognized as being due to extensive necrosis of the voluntary muscles. Olcott could find no lesions in the central nervous system.

In the meantime, the production of generalized muscular lesions by dietary means had been achieved by other workers in different species. Goettsch and Pappenheimer (4) in 1931 described profound alterations of the skeletal muscles in rabbits and guinea pigs maintained upon a scorbutic diet, supplemented by adequate amounts of orange or tomato juice and treated with ethereal ferric chloride to destroy the vitamin E (Waddell and Steenbock, 5)

They proposed for this disease the now generally accepted term "nutritional muscular dystrophy" Other species have been found to be susceptible to a similar disorder, sheep and goats (Madsen, McCay and Maynard, 6), ducks (Pappenheimer and Goettsch, 7), the tree kangaroo (Goss, 8), mice (Pappenheimer, 9), and hamsters (10) Symptoms suggesting muscle dystrophy have been noted by Anderson, Elvehjem and Gonce (11) in pups bred of vitamin E deficient mothers, but no studies on the pathology have yet been reported Dogs with chronic biliary fistulae have been found by Brinkhous and Warner (12) to develop muscle lesions, and these have been ascribed to failure of absorption of vitamin E when bile is excluded from the intestine

Chicks only exceptionally develop muscle lesions on an E deficient diet (Pappenheimer, Goettsch and Jungherr, 13, Glavind, 14)

Abundant evidence has accumulated to prove that this selective necrosis of the muscle fibers results from a deficiency of vitamin E in the diet It has been shown that vitamin E in the form of natural or synthetic alpha-tocopherol prevents or cures the disease in rabbits and guinea pigs (Shimotori, Emerson and Evans, 15, Mackenzie and McCollum, 16, Eppstein and Morgulis, 17) The minimal requirement of rabbits for dl-alpha-tocopherol acetate has been estimated by Eppstein and Morgulis on the basis of curative experiments at about 0 32 mgm per kilo per day, somewhat less than the figure of 0 6 to 1 06 mgm given by Mackenzie, Levine and McCollum (18)

The paralysis of suckling rats derived from vitamin E deficient mothers can also be prevented by the administration of natural or synthetic alpha-tocopherol, 0 5 mgm fed on the 15th to the 17th day affording protection to at least 85 per cent of the young (19, 20) The nutritional myopathy of ducklings is prevented by daily doses of 2 to 4 mgm of synthetic dl-alpha-tocopherol (21)

Two factors have been much discussed as to their importance in the production of experimental muscular atrophy

The first is whether cod liver oil in the diet of herbivorae exerts a direct toxic effect upon the skeletal muscles or whether it favors the dystrophic change by destroying, through its rancidity, vitamin E in the intestinal tract The first point of view was maintained by Madsen, Maynard and McCay (6), and by Davis, Maynard and McCay (22), who clearly demonstrated that the addition of cod liver oil expedites and intensifies the muscle lesions Nevertheless, the complete omission of cod liver oil from the experimental diet does not entirely eliminate the injury Goettsch and Pappenheimer (4), Madsen (23) and Mackenzie and McCollum (24) have shown that muscular dystrophy can be produced in guinea pigs with diets containing no cod liver oil This would seem to exclude toxicity of cod liver oil as a primary factor in the production of the lesions Mattill (25, 26), demonstrating the vulnerability of vitamin E to oxidation in the presence of unsaturated fatty acids, strongly questions the assumption of a direct toxic action In a recent paper, Mattill and Golumbic (27) bring additional evidence that the effect of cod liver oil is to favor the destruction of vitamin E through the development of rancidity in the gut

Another question which may be regarded as settled is the alleged participation

of a water soluble, not further identified member of the vitamin B complex in the deficiency   A dual deficiency was first postulated by Morgulis and Spencer (28)   At that time pure preparations of alpha tocopherol were not available   Further work on the prevention and cure of the disease in both rabbits and guinea pigs has convincingly excluded the complicity of a deficiency of a water soluble B factor in the causation of the lesions

It was recently reported by Holmes and Pigott (29) that orally administered thiamin chloride was effective in curing the suckling paralysis of rats   The authors appear to have been unfamiliar with the spontaneous regression of the lesions after weaning   Terry (30) has proven the ineffectiveness of vitamin B_1 in the prevention and cure of suckling paralysis

CHEMICAL STUDIES   1 *Changes in electrolytes*   Fenn and Goettsch (31) analyzed dystrophic rabbit muscle for K, Na, Cl, Mg and Ca   Potassium and magnesium were found decreased, sodium was increased out of proportion to gain in chloride, from which it was concluded that it entered the cells in exchange for potassium and magnesium   High concentration of Ca and total P were found in muscles showing histologic evidence of calcification of the fibers

A similar study by Morgulis and Osheroff (32) confirmed the loss of potassium and the increase in the sodium and chloride content   The calcium was also increased   The interpretation of these over all changes in electrolyte content is difficult because of the complex and varying composition of dystrophic muscles as regards normal, necrotic, and regenerating fibers   wandering cells, interstitial fat and connective tissue, and pathologic calcification of dead fibers

2 *Phosphorus compounds*   Morgulis and Spencer (33) found a decrease in total and soluble phosphorus and fractions thereof   Goettsch, Lonstein and Hutchinson (34) analyzed normal and dystrophic rabbit muscle for total phospholipid, total acid soluble phosphorus and total inorganic orthophosphate phosphorus   No striking changes were found until the muscle became severely degenerated   No change was detected in the total phospholipid phosphorus. Total acid soluble, and total inorganic phosphorus were decreased except in the presence of calcification, in which case, as might be predicted, the total acid soluble and total inorganic phosphate were increased   The phospho-creatin content of degenerated resting muscle was reduced absolutely, but was unchanged relative to total acid soluble phosphorus

Lu, Emerson and Evans (35) have also determined various phosphorus fractions of dystrophic rat muscle, as compared with muscle of rats on normal diet   There was a marked increase in total P and total acid soluble P, no change in creatin phosphate   a slight decrease in organic phosphate   The ability of the affected muscle to phosphorylate glycogen was reduced by about 40 per cent as compared with normal muscle

There is nothing in these studies to indicate that the described changes in phosphorus fractions precede the onset of lesions, nor do they offer any clue as to the intimate nature of the disturbed metabolism

3 *Lipids*   Morgulis, Wilder, Spencer and Eppstein (36) studied the lipid content of various tissues of rabbits with muscular dystrophy   They found no

changes in the heart and various internal organs, but the skeletal muscles showed a great increase in fat, lipoid P and especially in cholesterol (100 to 350 per cent) The rise in cholesterol preceded that of total lipoid P and fat, and must be regarded as a "specific characteristic" of dystrophic muscle, the proportion of esters to free cholesterol also increasing as the dystrophy progresses   There is an accompanying hypercholesterinemia (33)

4 *Creatin*   A number of workers (37, 33, 38) have demonstrated a reduction in the creatin content of dystrophic muscle of guinea pigs and rabbits, and of old and young rats (39, 40)   An increased urinary excretion of creatin in vitamin E deficient rats was reported by Verzár (41), and in rabbits by Morgulis (42)   Advantage has been taken of this creatinuria in following the progress of the disease in rabbits, its disappearance under vitamin E therapy has been a valuable test of the efficacy of the treatment (43, 44)

5 *Oxygen consumption*   One of the most interesting and perhaps significant alterations in the biochemistry of dystrophic muscle is the increased O_2 consumption   Victor (45), using a modified Warburg technic with Fenn volumeters, was able to demonstrate an average increase of from 200 to 400 per cent in rate of O_2 consumption per gram per minute when dystrophic rabbit muscle was compared with normal muscle from animals on a stock diet   Madsen (23) repeated these experiments with muscle from both rabbits and guinea pigs, and fully confirmed Victor's observations   Friedman and Mattill (46) likewise found an increased O_2 uptake in dystrophic muscle of rabbits when compared with that of animals on Purina rabbit chow, and a similar increase of more than 40 per cent was obtained in the muscles of 5 months old female rats kept since weaning on E deficient diet   At 13 months, when the rats had severe paralytic symptoms, the difference tended to disappear, and the Q O_2 was hardly above normal   A few preliminary experiments indicated that the administration of 5 mgm of dl-alpha-tocopherol acetate was followed by a fall in O_2 consumption within 24 to 120 hours

Houchin (10) has made similar observations in the hamster   An average Q O_2 of 1 70 was obtained in the semitendinosus muscle of normal control animals   The Q O_2 of degenerated muscle was as high as 4 55   After therapeutic doses of alpha-tocopherol, normal values were reached within 30 hours

A recent preliminary report by Houchin and Mattill (47) adds the very important observation that water soluble alpha-tocopherol phosphate dissolved in Ringer-Locke solution, brings about a reduction of 41 3 per cent and 35 7 per cent respectively in the O_2 consumption of dystrophic rabbit and hamster muscle   They believe that the alpha-tocopherol participates in a biological system, and does not act merely as a chemical antoxidant   The metabolism of normal muscle was not affected by the addition of alpha-tocopherol, so that it may be assumed that it already contains the optimal amount

All the preceding studies on O_2 consumption have been made on dystrophic muscle in which the interpretation of the results may be complicated by the presence of inflammatory or regenerating cells, or a variable proportion of fibrous or adipose tissue   Usually the metabolism of muscle from animals on a

stock diet of very different composition from the experimental one has been taken as a normal standard of reference   It therefore seemed important to find out whether a rise in the *in vitro* O_2 consumption on vitamin E deficient diet occurred also in the absence of morphologic changes in the muscle, and whether the administration of alpha tocopherol, without other modification of the diet, would inhibit this rise   The problem has been taken up by Kaunitz and Pappenheimer (48)   The skeletal muscle of young rats born of vitamin E deficient mothers has been found to show a significant increase in Q O_2 as compared with litter-mates which had received a single dose of dl alpha tocopherol acetate on the 15th day, and this increase occurred not only in dystrophic muscle, but also in the absence of lesions   The pectoralis muscle of chicks on an E deficient diet was also studied.   In this species, lesions of the muscles occur only exceptionally and were completely absent in the material examined   The findings were comparable to those of rat muscle

These observations complement those of previous observers by demonstrating that the increased O_2 consumption reflects an altered metabolism of the muscle tissue itself, and is independent of visible alterations in structure

6   *Total O_2 consumption of intact animals on vitamin E deficient diet.*   This has been little studied   Wood and Hines (49) discovered no difference in the cubic centimeter O_2 per M^2 of surface area per hour, between guinea pigs on Goettsch-Pappenheimer dystrophy-producing diet 13, and on a stock diet of grains, alfalfa and lettuce

Kaunitz and Pappenheimer (48) have followed the total O_2 consumption of rats from the 10th to the 150th day   As controls, in addition to rats on a stock diet (Rockland pellets), litter-mates were given a single dose of 1 mgm of dl-alpha-tocopherol on the 15th day, completely protecting them from the lactational paralysis   The O_2 consumption measurements were carried out in a closed curcuit apparatus at 29°C, the animals were maintained in a warm room at about the same temperature   Determinations were made at 1 or 2 day intervals during the early growth period, and weekly thereafter   Results were calculated as cubic centimeters O_2 per kilo per minute, and as cubic centimeters per M^2 surface per minute (Lee formula), both on basis of weight and age   From the time of weaning until the 70th to 80th day, or until the rats had attained a weight of about 100 grams, the oxygen consumption of rats which had received a single protective dose of dl alpha tocopherol during the lactational period remained consistently and significantly lower than that of their litter mate controls   After this time there was no appreciable difference

During the paralytic period, from the 18th to the 23rd days, the affected rats usually lost weight, probably because of inability to nurse, and their subsequent growth was retarded as compared with treated litter mates.   To determine whether the subsequent increase in Q O_2 was due to this transient weight loss, rats on a complete stock diet were subjected to partial starvation from the 17th to the 21st day   This resulted in a temporary depression in total O_2 consumption, but this was rapidly ironed out, and after the 21st day there was no significant difference between the starved rats and their litter mate

controls   It would seem therefore that the effect of the tocopherol in lowering the total oxygen consumption throughout the adolescent period is a specific one, and that transient inanition during lactation *per se* does not influence the subsequent O_2 consumption

The question may be raised at this point whether the increased metabolism in the vitamin E deficient animals is to be attributed to the altered metabolism of the muscle tissue, or whether it is possibly an indirect effect mediated through the thyroid   The literature bearing upon the condition of the thyroid in vitamin E deficiency is rather conflicting   Singer (50) reported the thyroid to be histologically hyperplastic in female rats which had been on an E deficient diet for 12 to 18 months, and Barrie (51) observed the same thing in the young of vitamin E deficient mothers   Telford, Emerson and Evans (52) were unable to confirm Barrie's findings, and Morgulis (53) states that the relative dry weight of the thyroid in dystrophic animals on a vitamin E diet is unchanged   On the other hand, Biddulph and Meyer (54) found a two-fold increase in the weight of the thyroid in male rats after 6 months on vitamin E deficient diet, as compared with those receiving wheat germ oil or stock diet   The addition of iodine to the E deficient diet restrained the thyroid weight to normal limits In the female they found no difference   In a second paper (55) hypertrophy of the thyroid is brought into definite relation with the low iodine content of the experimental diet and drinking water   The addition of wheat germ oil to the diet, without iodine supplement, alone reduced the thyroid weight and basal metabolism to normal limits

It is obvious that the increased O_2 consumption noted by Kaunitz and Pappenheimer during the period from 1 to 3 months cannot be ascribed to increased thyroid activity, since it is inhibited by alpha-tocopherol without other supplement   Moreover, the Hawk-Oser salt mixture in their experimental diet contains abundant iodine   The direct effect of alpha-tocopherol phosphate *in vitro* upon the muscle metabolism, as described by Houchin and Mattill, is further evidence against participation of endocrine glands

PHYSIOLOGICAL STUDIES   There have been few studies of muscular functions in vitamin E depleted animals   Victor (45) has determined the chronaxie and rheobase in dystrophic rabbit and duck muscle, and found them both significantly increased   Knowlton and Hines (39) have studied the functional capacity of the gastrocnemii of adult rats on a vitamin E deficient diet   The animals were survivors of offspring of vitamin E depleted mothers   The maximum isometric tension developed by the intact gastrocnemius in response to condensor discharges at 50 per second, was found to be definitely less than that of control animals receiving wheat germ oil   Histological examination of the muscle showed hyaline necrosis in only a few fibers, so that the functional alteration appeared not to be closely correlated with visible changes in structure In a subsequent paper, Knowlton, Hines and Brinkhous (56) demonstrated that synthetic dl-alpha-tocopherol, like wheat germ oil, favorably affected the muscular strength   Both preventive and curative experiments were made

Effect of inactivity   Section of the sciatic nerve or of the Achilles tendon in

young rats born of vitamin E depleted mothers has been found to protect the corresponding gastrocnemius from becoming dystrophic, provided the operation be performed before the 17th day (57, 58) The significance of this observation is not clear, but it does suggest that vitamin E is in some way implicated in the contractile phase of muscle metabolism rather than in the resting phase

SMOOTH MUSCLE   There is little to indicate that vitamin E deficiency has a profound effect upon non-striated muscle Goettsch and Pappenheimer (4) found the smooth muscle of intestine, bronchi blood vessels and uterus unaffected in rabbits and guinea pigs In female rats, however, there does occur, after long-standing vitamin E deficiency, a very striking brown coloration of the uterine musculature, not only in animals which have had repeated fetal resorptions, but in virgin rats as well (59, 60, 61) The pigment accumulates within the muscle fibers and interstitial cells, and may lead to degeneration and fibrous replacement It does not give an iron reaction, but hemosiderin does occur in the mucosa and interstitial tissue after repeated resorptions (Hessler, 62) The reactivity of the uterine muscle to various pharmacologic agents— acetyl-choline pilocarpin, prostigmin, adrenalin, ergotamin—was found by Hessler not to be altered The source and chemical nature of the pigment have not been satisfactorily determined

The only animal thus far studied in which smooth muscle appears to be selectively affected is the turkey, in which vitamin E deficiency regularly leads to necrosis of the gizzard musculature, with subsequent replacement fibrosis (63)

NERVOUS SYSTEM   Because of the large array of clinical papers which have appeared during the past two years dealing with the effects of vitamin E therapy in certain diseases of the central nervous system, it seems desirable to review with some care the experimental work upon which this treatment has been grounded

We have already referred to the paper of Lipschütz (2), who studied the brains and cords of young rats with suckling paralysis supplied by Evans and Burr He described (on the basis of Marchi preparations) degenerative changes in a the crossed and homolateral, descending and ascending vestibular tracts, b, the columns of Goll and Burdach, c, the tecto-spinal tract With Nissl preparations, it was possible to detect three or four stages of degeneration of the medio-ventral and lateral anterior horn cells, which alone were affected,— vacuoles, diffuse coloration of the cytoplasm, loss of nucleus and Nissl substance These changes were accompanied by neuroglia proliferation

Lipschütz attributed the paralytic symptoms in suckling rats entirely to these neurologic lesions having failed to recognize the presence of severe changes in the skeletal muscles   These were first described by Olcott in 1938 (3), and later by Pappenheimer (64), Barrie (65), Verzár (41), Demole and Pfalz (66) and by Evans and collaborators (67, 68) Olcott and Pappenheimer were unable to find any changes in the central nervous system

Undoubtedly the most thorough study of the central nervous system in vitamin E deficiency is that of Einarsen and Ringsted (69) published in 1938   This is invariably taken as supplying the experimental ground work for the therapeutic use of vitamin E in amyotrophic lateral sclerosis and other chronic

nervous disorders (70–91)   Ringsted (92) in 1935 had observed certain clinical abnormalities of gait and behavior in adult rats that had been kept on vitamin E deficient diet for a prolonged period   The animals developed a waddling gait, with inco-ordination and spreading of the hind legs, hypesthesia and hypalgesia, roughening of the pelage and loss of hair   Some of the rats survived for 2 years in spite of the progressive paresis

The lesions found by Einarsen and Ringsted did not tally exactly with those described by Lipschütz   The first structures to be attacked, according to these authors, were the proximal parts of the posterior roots, and the proprioceptive and possibly also the uncrossed paths in the fasciculus cuneatus and fasciculus gracilis   Later, there appeared increasing degeneration in the motor cells of the anterior horns, to which was attributed the progressive atrophy of the leg muscles   It was doubtful whether the peripheral afferent fibers were affected, changes in the spinal ganglia were relatively slight   In several animals, clinically in the last stages of the disease, partial degeneration of the cortico-spinal tract was noted   No changes were found in the tecto-spinal tracts, nor in the nuclei of the medulla or mesencephalon

As a rule, the "first and largest site" of the process was in the lumbo-sacral part of the spinal cord   The process gradually extended upward to the thoracic and lower cervical cord as far as the 5th segment   In a few cases the cervical cord seemed most severely affected

The anatomical picture thus resembled closely a combination of tabes dorsalis and progressive muscular atrophy   In the few cases in which the pyramidal tract was affected, the lesions were regarded by Einarsen and Ringsted as analogous to those of amyotrophic lateral sclerosis   The muscle lesions were interpreted as secondary to the spinal cord changes—a simple, neurogenic atrophy with occasional increase in the sarcolemma nuclei

Monnier (93) has also described alterations in the central nervous system in adult rats on vitamin E deficient diet   His material consisted of 38 adult rats which had been maintained by Verzár on a diet of starch, casein, lard, salts, cod liver oil and brewer's yeast   Symptoms were observed after 9 to 10 weeks and became progressively more severe   Three of the animals died between the 14th and 23rd months, with a loss of weight of 40 to 70 grams in the final 12 days   In addition to the symptoms described by Einarsen and Ringsted, the rats displayed head tremor, dysmetria, diminution in olfactory and auditory sensation, trophic or vegetative disturbances, exophthalmos, urinary incontinence, alopecia and ulceration

The skeletal muscles showed segmental necrosis, multiplication of sarcolemma nuclei and invasion of mononuclear elements   These muscular changes were associated with partial demyelinization of the intra-muscular branches of the peripheral nerves, and with slight rarefication of the posterior columns of the cord   Later there occurred marked degeneration of the fasciculi of Goll and hyperchromatosis and sclerosis of anterior horn cells   Contrary to the findings of Einarsen and Ringsted, the pyramidal tracts were never affected

In this country, similar studies have been made by Gutierrez-Mahoney (94) on rats supplied by Dr Karl Mason   Young rats with paralysis, offspring of

vitamin E depleted mothers and surviving rats, maintained for over a year on the vitamin E deficient diet and comparable to the adult rats of Einarsen and Ringsted, furnished material for this work.

Emphasis is placed on changes found in the ganglion cells—hyperchromatosis of cytoplasm with loss of Nissl substance, thinning and elongation of cell bodies, or a second type of change characterized by diminution of stainable contents and vacuolization and phagocytosis   Both anterior and posterior horn cells, the nuclei of the medulla, Purkinje cells, the larger and smaller cells of the dorsal ganglia and the cells of the cerebral cortex were affected   Myelin stains disclosed alterations in the sciatic nerves—swelling, fragmentation, ball formation and loss of myelin   The anterior and posterior roots in the cauda equina and long conducting pathways were also involved   Changes were seen in the anterior and lateral tracts, in the posterior columns and to a lesser extent in the cortico-spinal pathways and the corona radiata and median forebrain bundles   Thus the lesions described by Gutierrez-Mahoney were even more severe and widespread than those noted by Einarsen and Ringsted

We have listed the various lesions described by these authors in some detail   It is plain that there are wide discrepancies in the findings, indeed, no two investigators agree as to the distribution of the lesions

Wolf and Pappenheimer (95) have recently reported on the results of their study of this problem   Their material included 19 rats on vitamin E deficient diet, comprising 11 adult males, 4 females killed after periods varying from 180 to 365 days, and 4 young   Control animals on stock diet, on a simplified complete diet, and on vitamin E deficient diet supplemented by wheat germ oil or by alpha tocopherol were taken for comparison   Through the courteous cooperation of Dr Karl Mason, rats from his colony exhibiting the characteristic signs of chronic vitamin E deficiency were also placed at their disposal

Aside from changes caused by technical artefacts, and present with equal frequency in the control material, they found the central nervous system unaffected in vitamin E deficient rats.

Whether this cardinal discrepancy between their work and that of previous observers is attributable to subtle differences in diet, to differences in technical procedure or to differences in interpretation, it is difficult to say   As regards diet, Einarsen and Ringsted's diet $\bar{V}$ was extremely low in fat, nevertheless they found that the addition of oleic and linoleic acid, in addition to 50 mgm of peanut oil daily, did not prevent the appearance of the lesions   The diets used by Lipschütz, Monnier and by Gutierrez-Mahoney were practically identical with those in use in this laboratory   It is beyond the scope of this review to discuss the technical pitfalls which may give rise to wrong interpretations in the histologic study of the central nervous system, but it seems probable that the diversity in the distribution and character of the reported lesions may be ascribed to variations in technique   One may also call attention to the lack of adequate control material for comparison   The proper controls in this instance—animals on vitamin E deficient diet supplemented by protective doses of alpha tocopherol were not studied by the authors cited

In view of these contradictions, it would seem that the therapeutic use of

vitamin E in amyotrophic lateral sclerosis and other degenerative diseases of the spinal cord rests upon a very insecure experimental basis

There remains the possibility that the primary effect of the deficiency is upon the motor end plates of the muscles, and here again there is no complete unanimity in the reported findings   Rogers, Pappenheimer and Goettsch (96) were unable to detect visible alterations in the terminal neurites or end plates of dystrophic guinea-pig muscles, and Pappenheimer (9) reported similar negative observations in young rats with severe muscle lesions   Telford (97), on the other hand, found a reduction in the number of end plates in severely damaged muscles, with a return to normal upon recovery   There is at least complete agreement that the necrosis of muscle fibers precedes any loss of neurites, and that the nerve endings may be morphologically normal even when necrosis of the muscle has taken place   The possibility that there may be functional alterations in the absence of demonstrable morphological lesions has not been ruled out

Lesions of the brain in chicks on vitamin E deficient diet   Under the designation of nutritional encephalomalacia, Pappenheimer and Goettsch (98), in 1931, described extensive ischemic necrosis occurring in chicks on a diet deficient in vitamin E

The first symptoms indicative of severe cerebral damage appeared between the 20th and 30th day after hatching—coarse tremors, ataxia, head retraction, propulsive, retropulsive or rotational movements, usually ending in death Some animals recovered and went on to normal development, a phenomenon comparable to the spontaneous regression of the lesions in the suckling paralysis of rats   The pathology is, distinctive   The lesions affect the cerebellum, cerebrum and medulla in that order of frequency and are essentially areas, often large and grossly visible, of ischemic necrosis   There is death of ganglion cells and glial elements, hemorrhage, edema and capillary thrombosis   Later, if the chicks survive, reparative changes take place (99)

The disease is preventable by various vegetable oils and by their non-saponifiable fractions after removal of the sterols (100), and by alpha-tocopherol, natural or synthetic, in daily dosage of 0 1 to 0 2 mgm (101, 13)   The disease can be produced on a complete diet in which vitamin E is destroyed by heating with ethereal ferric chloride (102), and it occurs as a spontaneous disease under field conditions (103)   Although the brain in vitamin E deficient chicks bears the brunt of the injury, the lesions cannot be interpreted as a specific toxic effect upon nerve tissue, since they are obviously the effect of vascular occlusion The pathogenesis of the lesions is still obscure, whether due to vascular spasm or to capillary thrombosis, but their infarct-like nature cannot be denied

The "alimentary exudative diathesis" described by Dam and his co-workers (104), and preventable by large doses of alpha-tocopherol, appears not to be correlated with the encephalomalacic condition   It has occurred very rarely with the standard diet 108 used in this laboratory, and prolonged extraction of the casein with alcohol and the low fat content of the diet seem to be, if not essential, yet favoring factors in its onset (105)

Mason (106) has recently called attention to the frequent occurrence of cu-

taneous and intracerebral hemorrhage in rat embryos of vitamin E deficient mothers. Subcutaneous edema, with or without muscle necrosis, occurs also in new born mice, born of vitamin E depleted mothers (9)   It would seem that in certain species and under certain conditions not yet defined, vitamin E deficiency is attended by circulatory disturbances, and perhaps by alteration in capillary permeability

GENERAL DISCUSSION   In concluding our review, we may summarize this analysis of the literature with the generalization that vitamin E plays an essential part in the metabolism of skeletal muscle in all species of mammals that have been investigated and in ducklings   It has, in our opinion, not been satisfactorily shown that vitamin E is essential to the integrity of the nervous system

There are many interesting and fundamental problems that demand further inquiry   The most pressing, it would seem, is to define in chemical terms the exact rôle that the tocopherols enact in the complex chemism of skeletal muscle. With active, water soluble preparations now available, progress along this line is inevitable   The observation that immobilization of muscle, whether by nerve section or tenotomy, offers protection against degeneration of the fibers, suggests that the vitamin is concerned with the contractile, rather than the resting metabolism of the muscle   The proven fact that the total O_2 consumption of the muscle of vitamin E deficient animals is reduced by alpha tocopherol, both *in vitro* and *in vivo*, should give a clue to further analysis of its action Very uncertain still is the importance of vitamin E in the nutrition of human muscle, although it is most unlikely that human muscle will be found to differ fundamentally in its requirements for this vitamin from that of other mammals As chemical tests are perfected and adapted to clinical use, dietary assays extended, and more is learned about the conditions which govern absorption and utilization, the rôle and importance of vitamin E in human muscle nutrition will become clearer

For other discussions of the relation of vitamin E to the neuro-muscular systems, the writer wishes to call attention to the recent excellent reviews of H M Evans (107), Wolbach and Bessey (108), and Karl Mason (106)

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MICRORESPIRATION TECHNIQUES

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Microrespirometer techniques have been developed in response to needs for methods of high sensitivity, rapidity of response, or both. The problems approached by these methods may, in general, be grouped into one of two categories

1 Investigations into the temporal distribution, over relatively long total times, of processes whose progression is mirrored in gas concentration changes Micromethods are needed for reactions producing a small total change   Stability and sensitivity are equally important

2 Investigations into the temporal distribution, over short total times, of processes whose progression is mirrored in gas concentration changes   Speed of response and sensitivity are at a premium whereas stability of the measuring instrument becomes progressively less important as observation time shortens

During the past forty years or so a number of procedures have been developed to deal with such problems as they have arisen in biological researches   It is the purpose of this review briefly to evaluate their relative sensitivities and precisions, and to point out their assets and limitations   The related problems of calibratory techniques and estimation of amount of metabolizing material will also be touched upon

METHODS OF HISTORICAL INTEREST   *The biometer*   Possibly inspired by the methods of Pettersson and Pettenkofer (1) (2), Tashiro, in 1913–14 (3) (4) (5) (6) (7), constructed an instrument to which he gave the name "biometer"   With it he demonstrated that surviving nerve produces carbon dioxide and that the rate of production is increased by stimulation

A nerve segment was suspended in a CO_2 free, glass respiration chamber Known amounts of gas could be transferred to an adjoining analysis chamber containing a drop of barium hydroxide whose surface was observed with a lens If a limiting amount, or more, of carbon dioxide was contained in the sample visible crystals of barium carbonate formed on the drop surface.   It had been determined that a "quite definite" amount of carbon dioxide had to be introduced into the test chamber to produce a just visible precipitate.   "By determining the minimum volume of any given sample of gas necessary to give the first visible formation of the precipitate, its carbon dioxide content could be estimated accurately, since this volume must have contained just the known detectable amount of carbon dioxide "

Controls indicated that the method was sensitive and quantitatively reliable For a given construction, the minimum detectable quantity of carbon dioxide was 1×10^{-7} grams   Thus the limiting sensitivity of the method calculates at 0 05 cmm

Table 1 compares the quantity of carbon dioxide found in 1 cc of gas with the known composition

The technique was criticised because data on nerve respiration gave values considerably higher than those found by others   Guttman (8) explained this disagreement in terms of a gush of carbon dioxide following injury to nerve (8) (9) (10) (11) (12)   The order of magnitude of the discrepancy, however, seems to invalidate this explanation   It is difficult to reconcile these facts with the results obtained in control calibration

The biometer, then, was a relatively sensitive instrument detecting 0 05 cmm of carbon dioxide produced in carbon dioxide free air   It achieved a fairly high degree of precision   When carefully sealed it was stable   Thus it was well suited to the use to which it was put, the detection of a small total change over a relatively long total time

The Winkler titration   The Winkler titration was adapted to biological experimentation as a method for following changes in concentration of dissolved oxygen   First used by Winkler (13) (14) in a study of oxygen solubility, the method has since been frequently revised and improved   Sources of error have been

TABLE 1

SAMPLE VOLUME REQUIRED TO GIVE PRECIPITATE	WEIGHT OF CO_2/cc IN GRAMS $\times 10^{-7}$		DIFF (J T)
	Found	Taken	
cc.			*per cent*
1 0	1	0 92	8
0 5	2	2 3	15
0 55	1 82	1 83	0 5
1 5	0 67	0 62	8
2 25	0 45	0 45	0
Average			6 3

pointed out, and modifications of the technique have been devised to deal with them (15) (16) (17) (18) (19) (20) (21) (22) (23)   For details one should consult the critical reviews of Theriault and of Theriault and McNamee, and of Allee and Oesting (17) (18) (19)

Lund, having used the method in a study of paramecium respiration (24) adapted it to micro-studies, and (25) was able to handle as little as 5–10 cc of solution   Thompson and Miller (26) designed apparatus making the micro-titration more convenient and accurate   Using their technique, Snoke (27) compared the micro- and macro-Winkler titrations obtaining the results reproduced in table 2

In 1933, Kawaguti (28) developed apparatus making possible the titration of as little as 0 1 cc of solution   Control experiments yielded the results shown in table 3

Though sources of error were not determined, Kawaguti suggested that corrections could probably be worked out so that the method might be applicable to as little as 0 1 cc of solution with accuracy

Allee and Oesting (19) have the following to say about the macro-method   "It

has been our experience that with usual contents of oxygen (N B The method is less accurate with very low total quantities of oxygen) we can be sure of repeatability to about 0 03 cc per litre Hyman's error, as determined by duplicate determinations, was 0 02 cc per litre When the animals being tested consume 2 00 cc of oxygen per litre the error inherent in the method amounts to from 1 0 to 2 5 per cent When, however, small differences are recorded the inherent percentage of error mounts " Allee found (29) that a modification, requiring 14 cc of solution was about as good as the macro method Putting these data together one can conclude that for the micro method, requiring 14 cc of sample,

TABLE 2

SAMPLE NUMBER	DISSOLVED OXYGEN			
	Macromethod, 250 cc. sample		Micromethod, 4.9 cc. sample	
	Average of 3 determinations	Maximum deviation	Average of 3 determinations	Maximum deviation
	mgm. per liter	mgm. per liter	mgm. per liter	mgm. per liter
1	7 46	0 07	7 42	0 07
2	9 93	0 05	9 91	0 04
3	9 59	0 05	9 64	0 06

(From Snoke—Ecology 10 163, 1929)

TABLE 3

VOLUME OF TEST FLUID	cc./L.	OXYGEN (MEAN OF MORE THAN 11 TESTS) (MAXIMUM DEVIATION)
cc		
0 5	4 64	0 06
0 4	4 58	0 05
0 3	4 50	0 03
0 2	4 45	0 06
0 1	4 48	0 08
2 0	4 89	0 08
macro Winkler on 200 cc sample	5 00	0 01

(From Kawaguti—J Fac Sci Imp Univ Tokyo (Zool sect) 3 183 1933)

to be good to 1 to 2 5 per cent the total change in oxygen concentration must be about 28 cmm or about 2 cmm per cc Kawaguti's datum on a 2 cc sample is also about 2 per cent off the value determined by the macro-method, but on a 0 1 cc sample the discrepancy is about 10 per cent

These data indicate that the best one can say for the micro-Winkler titration is that it is capable of detecting a change of 28 cmm or so of oxygen in a 14 cc sample of fluid with an error of 1 to 2 5 per cent Compared to other methods discussed, this is a low sensitivity In addition, the multiple sources of error and the long time required for an analysis make the method unsatisfactory It is being and will doubtless continue to be, largely replaced by the polarographic technique.

The indicator method   In 1916 Haas (30) developed an indicator method for following carbon dioxide production   Since the material producing CO_2 was to be submerged in a solution of an indicator it was essential that the indicator be non-toxic and slowly penetrating   Phenolsulphonephthalein was used   By a comparator method it was possible to measure hydrion concentration changes as small as 1 or 2×10^{-6} If the determinations were made in pure distilled water, free of carbon dioxide, changes as small as 2 or 3×10^{-8} could be detected   The method was used by Moore (31) (32) (12)

Osterhout (33) modified the technique by circulating the gas, in equilibrium with the solution being studied, through a solution of indicator   Osterhout and Haas (34), and Osterhout (33) (35), and Osterhout's students (Gustafson, Irwin, etc) modified and used the method in respiration studies

Lund (36) combined the indicator method with the basic construction of the biometer   Material to be studied was suspended above barium hydroxide containing phenolsulphonephthalein   At given times a known amount of the barium hydroxide solution was titrated with HCl and the CO_2, produced in the intervals, determined

Parker suspended tissue over barium hydroxide containing the indicator, and frequently renewed the absorbing surface   He was able (9) (37) (38) to confirm the findings of Tashiro on carbon dioxide production by nerve   The sensitivity of the method was estimated to be about one-tenth that of the biometer The error amounted to about 10 per cent

The biometer constituted a challenge to those investigators requiring some means of following CO_2 production by submerged tissues   The development of the indicator method answered the challenge with a measure of success   The latter technique was more widely applicable, simpler to use, and could be used for following rates of gas production, which was a more tedious process with the biometer   Osterhout and Haas (22) combined the indicator method with the Winkler titration, making possible simultaneous measurement of carbon dioxide and oxygen in solution

MORE RECENT METHODS   *The optical lever micromanometer*   The optical lever manometer has, for many years, been known to provide a convenient means of measuring relatively small pressure changes (39) (40)   As a macroinstrument it has been chiefly applied in physiological experimentation requiring precise responses to high frequency pressure changes (41) (42)

The incorporation of the optical lever into a micromanometer for biological use was first successfully attempted quite recently (43) (44) (45)   In 1939 Heatley, Berenblum and Chain described such an instrument sufficiently sensitive to measure gas volume changes of 1 cmm per hour

A paraffin lined chamber of 40 to 80 cmm ground into a glass plate is so shaped that droplets placed in its several arms may be maintained separate A steel ball, coated with plastic, is placed in one of the arms   With an electromagnet, this ball can be moved and used as a mixer   The vessel is constructed to allow filling with given gas mixtures   The plate which closes it carries an 18 μ thick mica sheet cemented over a central hole   On either side of the

opening there is another aperture which may be made to coincide with one of the arms of the chamber. A stream of gas can then be circulated through the chamber and the latter subsequently closed by rotating the cover

The reaction material and reagents are placed in the vessel and the top is closed. A bit of lubricant and a bracket seal and immobilise the mica-glass joint. The upper surface of the mica carries two small plane mirrors which reflect incident beams of light to an observation surface. As the mica sheet becomes depressed or bulges outward, due to absorption or production of gas within the chamber, the reflected beams are displaced. The applied pressure necessary to return the mirrors to their original positions may be manometrically measured, and attendant volume changes calculated
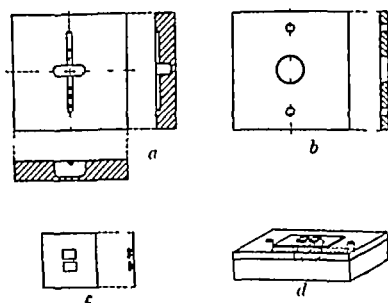
FIG 1 Components of respiration chamber *a* cup *b* plate ' *c* mica membrane with mirrors attached *d* complete assembly
(From Heatley Berenblum and Chain—Biochem J **33** 53, 1939)

Accuracy has been determined in several ways. The volume of carbon dioxide displaced from bicarbonate by a known amount of acid has been measured and compared with the calculated value. The results are reproduced in table 4

Precision was also tested by measuring the amount of oxygen liberated from hydrogen peroxide by catalase. The 'known' values were obtained by per manganate titration. The results obtained with the microrespirometer and with the Warburg are reproduced in table 5. In each case approximately 1 cmm of gas was evolved in the microrespirometer

The optical lever micromanometer does not possess the sensitivity of most of the other micromethods mentioned. Cumbersome and requiring considerable accessory equipment, it is chiefly notable for the convenient design of the cups allowing it applicability to all the types of problems to which the standard Warburg may be applied. Precision is about equivalent to that of the Warburg technique. A number (up to six for the present design) of the respirometers can be used at one time (45). Thermobarometric controls afford relatively high stability for long time measurements

TABLE 4

VOLUME CO_2 EVOLVED (CALC. VALUE = 0.90 CMM.)	ERROR
cmm	*per cent*
0 83	−8
0 85	−5 5
0 90	0
0 82	−9
0 87	−3 5
0 84	−6 5
0 87	−3 5
0 85	−5 5
0 90	0
0 96	+6 5
0 87	−3 5
0 89	−1
0 88	−2
0 89	−1
0 92	+2
Average	−2 7

(From Heatley, Berenblum and Chain—Biochem J **33** 53, 1939)

TABLE 5

Volume oxygen per cubic centimeter of hydrogen peroxide

MICRORESPIROMETER	WARBURG	CALCULATED
204		202
208		
198		216
200		
78		
77	78	
77	81	
77	81	
80		
81	77	
74	77	84 5
75	78	
79	79	
79	77	
85	80	82 5
79	81	
82		

(From Heatley, Berenblum and Chain—Biochem J **33** 53 1939)

The Cartesian diver   A considerable volume change occurs isothermally in systems in which certain chemical reactions take place (40)   Taking advantage of this fact, Linderström Lang (47) (48) and Linderström Lang and Lanz (49) (50) adapted the falling drop method for determining specific gravity to studies in enzymatic histochemistry   The technique was modified from that used by Barbour and Hamilton (51) in the estimation of specific gravity, and by Vogt and Hamilton (52) and by Fenger Eriksen, Krogh and Ussing (53) in the estimation of deuterium oxide

A floating object comes to rest in a fluid column of graded specific gravity at such a point that the specific gravities of the two are equal   This fact underlies both the gradient tube technique and the cartesian diver method   Though the gradient tube has been used only for the study of volume changes in liquid systems the cartesian diver has been used in microgasometry   A few words about the gradient tube seem appropriate here

In a vertical glass tube, at a constant temperature, a practically linear specific gravity gradient can be produced by properly mixing kerosene and bromo benzene   A droplet of a watery solution placed beneath the surface of the mixture which has been previously saturated with water at a suitable vapor pressure, will fall and find an equilibrium position where the specific gravity of medium and drop are equal   If, however, reactions occur within the drop, producing a volume change, the drop will not come to a standstill but will continue to fall or begin to rise at a rate which, under certain conditions, is a measure of those processes

The gradient in the kerosene-bromobenzene column is measured by noting the positions at which test drops of known density come to rest   Drop movement is followed with an ocular micrometer, and specific gravity is ascertained by comparison with the positions of standard drops   The drops being small and on all sides surrounded by fluid come almost immediately to temperature equilibrium

The accuracy of the density measurements is 1 to 3 in the sixth decimal place Since 0 1 cmm drops can be used, this corresponds to a volume change of about 1 to 3 $\times$ 10^{-7} cmm   which it is claimed, can be measured within a few per cent of error   Such a combination of extreme sensitivity and high precision has been demonstrated for none of the other techniques mentioned in this review

First described in terms of its usefulness in biological experimentation by Linderström Lang (54), the cartesian diver technique has been extended by Linderström Lang and co-workers and by Boell and co-workers (55) (56) (57) (58) (59) (60) (61) (62) (63)

A glass vessel (diver) of some 10 to 30 (occasionally up to several hundred) cmm is stoppered with an oil droplet free to move in the neck of the vessel Submerged in a fluid medium the vessel quickly comes to equilibrium position Should the inside pressure change, the oil droplet and outside fluid respond by moving in the neck, and depending on whether total volume increases or decreases the vessel rises or falls in its external medium   The pressure which

must be applied to return the diver to its original position can be measured and translated into volume change

In his original paper Linderstrom-Lang stated that "any variation of the quantity of gas in the diver may be measured with an accuracy of $\dfrac{10 \times 0\ 02}{100}$ cmm "

(54)   Boell, Needham and Rogers set the sensitivity at 0 001 cmm
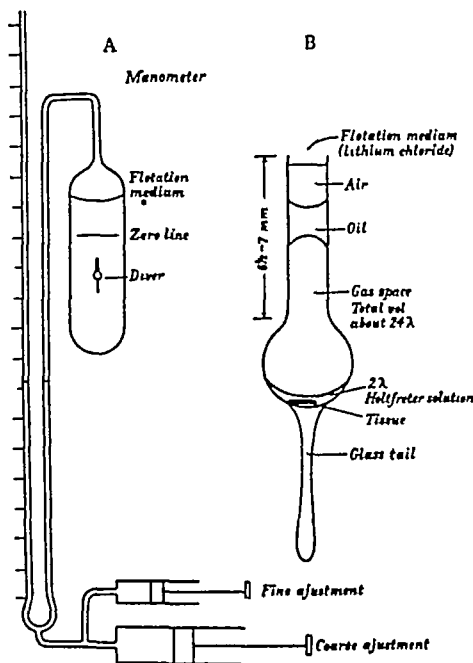
FIG 2

(From Boell, Needham and Rogers—Proc Roy Soc London **B 127** 322, 1939)

The equilibrium pressure is said to be reproducible within 1 to 2 mm of water (the diver can be made to stay at the mark for ten seconds or so)   If v be the gas volume of the diver and p the equilibrium pressure, then

$$\frac{\Delta v}{v} = \frac{\Delta p}{p}$$

Since p and v are of the order of magnitude of 10,000 mm and 10 cmm respectively, an accuracy of pressure reading of 1 to 2 mm of water corresponds to an accuracy of volume measurement equal to 0 001 to 0 002 cmm   From this one would expect that, were the total volume change only 0 001 to 0 002 cmm , the error to be expected to its immediate measurement would be about 50 to 100 per cent

Boell, Needham and Rogers studied cystein oxidation and compared diver measurements with Warburg results   They state that "in the case of cystein oxidation rate the average diver results were 106 per cent of the Warburg results, in the case of cystein oxidation end values they were 99 per cent, and in the case of yeast fermentation 102 per cent"

These same workers have published a graph relating oxygen uptake by cystem oxidation to time   Though only approximately, oxygen uptake can be tabulated against time as read from the points on this graph   If such an approach is not too crude one may conclude that, for gas volume changes of an average of 0 0026 cmm *each* reading of such a volume change may, on the average, be 70 per cent inaccurate   This conclusion would be in approximate agreement with the estimate of Linderström Lang already cited   On the other hand, when the volume change between readings is of the order of 0 04 cmm the deviation is considerably less.

The technique is thus seen to be quite sensitive (to about 0 002 cmm)   The inclusion of thermobarometer divers allows an experiment to be continued over many hours with a high degree of stability   For minute to minute measurements of volume changes of the order of 0 001 cmm precision is poor   Interpolation of such volume changes from larger total changes is very accurate   It is probable that with a smaller diver than that which is commonly used, and with the exercise of extreme care in setting the diver at the reference mark greater accuracy in the immediate estimation of very small volume changes could be attained

The polarograph   The studies of Kucera, in 1903 (64), on the electrocapillary curve of mercury led to the development of the polarograph by Heyrovsky (65) and by Heyrovsky and Shikata (66)

The method depends on the fact that solutes (electrolytes or non-electrolytes) are reduced (or oxidized) when a current is passed through their solutions at voltages above their decomposition potentials   The current flowing at such a potential (half wave potential) depends on the rate of diffusion of the solute to the electrode, and this rate depends, in turn, upon its concentration   Current measurements give quantitative data on solute concentration

In the usual polarographic determination the dropping mercury electrode is used   For details of the method and for the theory underlying it one should consult the work of Kolthoff and Lingane (67) (68)

The technique has been applied to the estimation of oxygen in solution and is far superior to the Winkler titration   The possibility of thus using it dates back to work of Vitek in 1933 (69)   It has since been used in a number of biological studies (70) (71) (72) (73) (74)

Objection to using the method with living tissue has stemmed chiefly from the fear that metallic mercury in contact with the medium might poison the material being studied   In 1940, Kolthoff and Laitinen (75) showed that oxygen in solution can be polarographically determined using a platinum wire microelectrode in place of the dropping mercury electrode.   Papers of Laitinen and Kolthoff (76) (77) published in 1941 should be consulted for detail

Davies and Brink (78) described a microrespirometer incorporating the platinum microelectrode.   A bright platinum or platinum iridium wire (about 27 μ in diameter) is placed in circuit with a calomel half-cell by way of a glass tube containing tissue and its medium   The platinum electrode is made 0.9 volt negative with respect to the half-cell, and the current which then flows is

proportional to the oxygen concentration of the fluid   A sensitivity to 0 001 cmm volume change of oxygen was attained

The stationary platinum electrode does not come immediately to equilibrium with the solution in which it is placed   Current intensity gradually changes for from two to ten minutes (75) (76) (77) (78)   In certain instances this defect has been overcome by rotating the electrode (75) (77)

The prospect of even greater sensitivity, great speed of response if the "seeking" electrode is close enough to the source of changing oxygen concentration, relative indifference to temperature changes, high precision, and convenience of operation make this instrument one which will probably have very wide applicability in microrespirometry   The electrode has also been directly inserted into the tissue being studied (78a)

The capillary microrespirometer   The capillary microrespirometers to be discussed are all built, more or less, about the same basic design [1]   Essentially there are two chambers, one of which contains the biological material, its medium and specific reagents for the absorption of certain gases   The other contains medium and reagents alone   The two chambers are connected by capillary tubing carrying a fluid index droplet   Gas volume change in the tissue chamber creates a pressure difference along the capillary lumen, in response to which the index drop moves   Droplet movement is recorded and timed, and these data are translated into volume change

First used by Thunberg in 1905 (79) (80) (81), the capillary micromanometer was a gas analysis apparatus of the Pettersson type (1) permitting introduction of the animal or tissue into the measuring pipette   Change in volume of enclosed air indicated the difference between oxygen absorbed and carbon dioxide produced   The air was then passed over potassium hydroxide for carbon dioxide absorption   Later, Thunberg (82) (83) modified the apparatus using equal volume respiration and compensation chambers, each about 3 cc , with a capillary of 0 2 to 0 1 mm diameter   This was the first instance of the use of a compensation chamber in a respiration apparatus   The movement of a petroleum index droplet was observed by the naked eye against a millimeter scale An upward convex capillary allowed the index to come always to the same equilibrium position   Modified by Winterstein (84), the apparatus was used in further respiration studies   The ratio of compensation to respiration chamber volume was unity   A volume change of about 1 4 cmm could be measured Nothing was recorded concerning regularity of movement of the index   During the next few years the apparatus was used in a number of laboratories (85) (86) (87)   In 1912–14 Winterstein (88) (89) (90) again modified the apparatus by incorporating a mercury manometer

The index being set at zero the level of the mercury column was noted   When

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[1] The Barcroft and Warburg manometric techniques and the variations thereof are not being considered in this discussion, because they have been so carefully and exhaustively treated by Dixon in his book on *Manometric Methods* (Cambridge University Press, 1934) A further development was described by Summerson in the Journal of Biological Chemistry vol 131 (1939), p 579

a reading was to be taken the droplet was brought to zero position by means of a screw clamp This simplified things considerably It was not necessary to know chamber volumes or capillary diameter to translate to gas volume change A somewhat later model than the one described, allowing the chambers to be filled with any given gas mixture, was used by Wolf (91)

Krogh (92) (93) (94) (95) modified the Barcroft blood gas apparatus, in 1914, to serve as a micromanometer Chambers communicated by way of a 0 4 to 0 5 mm internal diameter U tube The index was kerosene and the manometer itself was outside the thermostat Only the "reinste Petroleum" was used, but nothing was said about regularity of meniscus movement for small pressure changes Krogh stated that the Winterstein apparatus could ' be made still more sensitive but (it) is not as well suited to prolonged experiments" as his own This comment stems from the fact that the Winterstein apparatus incorporated a very short capillary, hardly a serious difficulty

In 1920, Adam (96) combined Winterstein's mercury burette with the Krogh manometer Paraffin oil of B.P. 160° to 190°C served as index. Volume changes of 20 cmm per hour could be measured with 2 to 3 per cent accuracy Bass (97) introduced the horizontal microscope for following the index. Bodine and Orr (98) employed a slightly modified Krogh manometer

In 1925 Novy, Roehm and Soule (99) made the next improvement in this type of microrespirometry Up to that time the ratio between compensation and respiration chamber volumes had always been close to unity Novy, however, pointed out that "Sensitivity of the closed end manometer varies directly with the volume of air in the sealed end and inversely as the square of the radius of the manometric tube" A gain in micromanometric technique, this modification was not taken advantage of until some years later when Gerard (99a) utilised the same principle in the construction of a modified Warburg apparatus

Fenn (10) devised a capillary micromanometer which he himself then found to be (100) "essentially similar to Thunberg's original design" Though the principle of large compensation chamber opposed to smaller respiration chamber was not incorporated, the instrument has been used by many investigators The smallest capillary was about 0.3 mm i d Meniscus position was read to 0 1 mm with a hand lens so that the smallest appreciable volume change was about 0 03 cmm In 1935 Fenn wrote a detailed paper on the theory and technique of the method (100)

The differential volumeter was now used by a number of workers (101) (102) (103) (104) (105) (106) (107) (108), but none of these described any attempt to improve sensitivity Very small movements of the index were not measured, and neither irregularities in the movement of the droplet nor deformity of the meniscus became important Consequently no detailed study of index fluid was made

In 1933 Schmitt (109) further developed the capillary microrespirometer to a point where it was sensitive to a 0 0005 cmm volume change A large compensation chamber was not used though both vessels were of relatively large absolute volume Projected experiments made it desirable to make observa

tions on index movements over very short periods of time   To locate the droplet a traveling horizontal microscope was used   Considerable attention was paid to the selection of index fluid   After trials with dimethyl aniline, butyric acid, valeric acid, and others, highly purified kerosene was finally used for the majority of experiments   Even so there were occasional difficulties due to residual resins

It was next found that to get a smoothly moving droplet, over periods of time as short as three minutes, it became necessary to go to extremes in temperature control   Finally, with a thermostat accurate to "better than 0 001°C " a smoothly moving meniscus was observed   For 10 mgm of nerve (wet weight) readings were consistent to 2 to 3 per cent even if made as frequently as every two minutes   Such precise thermoregulation was probably necessary because of the relatively large chambers used and by their rather wide separation in the bath

Schmitt pointed out that basal drift of the index might be due to slipping at ground joints, or to unequal evaporation of the droplet at its two surfaces Jamming the stoppers and wetting the capillary walls before closing the bridge cock, made it possible to diminish equilibration time to half an hour or less Capillary diameter was 0 4 to 0 5 mm

Thus with a 16 mm objective and a 6× ocular, the apparatus was sensitive to a volume change of 0 0005 cmm   Precision to 2 to 3 per cent with a total volume change of about 0 02 cmm was attained   This volumeter has also been used by Hill (110)

A capillary microrespirometer of different design has been described by Stefanelli (111) (112)   The chief innovations were a reduction of chamber size to 80 cmm , and the incorporation of a glass window to allow observation of tissue   The instrument was sensitive to a volume change of 0 003 cmm

Howland and Bernstein, in 1931 (113), studied the respiration of a single paramecium by the capillary method   It is interesting that with their comparatively crude apparatus they found the oxygen consumption of a single Paramecium caudatum to be about 0 0005 cmm per hour, and it was only in 1940 that Boell and Woodruff (62) with the cartesian diver technique, determined the value for the Paramecium calkinsi to be 0 00048 cmm per hour per organism

The chief importance of the work of Bernstein and Howland is derived from the finding that if one heats capillaries sufficiently to seal them they require many hours to cool   This is, of course, also dependent upon the efficiency of the thermostat employed and the thickness of the capillary walls   The point is pertinent concerning the earlier work of Kalmus who (114) (115) made respirometers by drawing tubing out to capillary dimensions at one end   The material to be studied was sucked in and followed by a drop of paraffin oil   The end was heat sealed   The fine capillary tip was next thrust through the surface of a 10 per cent KOH solution and then into paraffin oil   The interface between the alkali and oil was followed with an ocular micrometer   This work must have been highly unprecise due to the effect of passing the measuring

capillary through a fluid surface with its adsorbed contaminants   The same
objection can be raised to the work of Pearce and Gerard (116)

In 1934 Gerard and Hartline used both a very small respiration chamber
(about 60 cmm volume) and a very large compensating chamber (117)   The
small respiration chamber greatly increases stability   Drop movement irregu
larities are due, among other things to temperature fluctuations   Thermal
movement of the droplet is minimized as volume is decreased, since in this case
volume change is proportional to total volume   To prevent drop movements
due to changes in atmospheric pressure the manometer is closed off from the air
Instead of achieving this by balancing the respiration with an equal volume com
pensation chamber, these workers enclosed the entire measuring capillary in a
jacket of relatively large volume   Thus sensitivity was not sacrificed for sta
bility, and the achievement of the latter was, at the same time, simplified   The
ratio of control volume to respiration chamber volume being about 1000 1 the
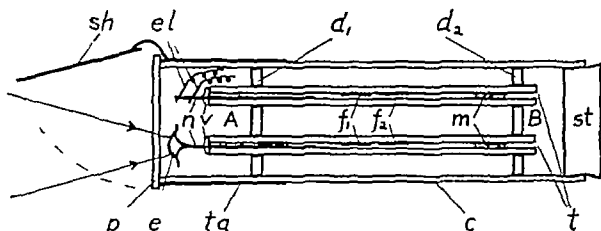
Fig 3 f_1 and f_2—filter papers with acid and alkali m—index droplets v—vaseline seals
n—tissue st—rubber stopper
(From Gerard and Hartline—J Cell and Comp Physiol 4: 141 1934)

volume change represented by any given droplet movement was effectively the
completely undamped volume change (the correction factor being 1/1000)

Temperature control was found to be simplified much beyond the elaborate
apparatus employed by Schmitt   Capillaries of 0 5 to 1 1 mm internal diameter
were used with a droplet of isotonic NaOH as index   Index movement was
followed by means of a horizontal microscope   Volume sensitivity achieved
was about 0 0013 cmm   For minute to minute figures to be reproducible the
total volume change had to be about 0 02 cmm   Reproducibility was therefore
just as good with this simple technique as with the apparatus of Schmitt

Since this work the basic design of the instrument has been used by a number
of workers some of whom have modified and adapted it to special problems (118)
(119) (120) (121) (122) (123) (124) (8) (116)

Recently several attempts have been made to incorporate some sort of device
with the capillary micromanometer so that reagents could be mixed during
an experiment

Thimann and Commoner (125), in 1940, made a contribution toward this
end   Using small capillaries (0 2 to 0.3 mm i d) they arranged the apparatus

to allow for the addition of substrate during an experiment   Measuring droplet movement to 0 1 mm these authors attained about the same sensitivity as previous workers   However, reading the meniscus to the nearest 0 1 mm means an average error of 10 per cent per mm of movement or 0 006 cmm for a volume change of 0 060 cmm   Thus for volume changes of 0 006 cmm (which is of the order of magnitude of sensitivity of most of the constructions discussed) the error would be about 50 per cent, on the average, and for volume changes of 0 001 cmm it would be about 400 per cent   This sort of error could easily be obviated by more accurately determining meniscus position

Cunningham and Kirk have made an effort in the same direction (126)   Side by side, in a brass block, there are placed respiration chambers which, by way

TABLE 6

VOLUME CO₂ LIBERATED (CALCULATED)	VOLUME CO₂ LIBERATED (MEASURED)	ERROR
cmm	*cmm*	*per cent*
2 60	2 37	−7
2 60	2 48	−5
3 00	2 72	−10
3 15	3 12	−3
2 98	2 85	−4
2 38	2 46	3
2 95	3 01	2
2 95	3 01	2
2 95	3 01	2
2 16	2 09	−3
2 16	1 99	−8
2 16	2 08	−4
3 42	3 60	5
2 16	2 08	−4

Average per cent error   = ±4 5
Average per cent recovery = 98 0

(From Cunningham and Kirk—J Gen Physiol 24 135, 1940)

of hollow vertical pillars, communicate with each other through a glass capillary Kerosene serves as index fluid   The authors state that "The symmetrical construction and the placing of the chambers inside a metal block insure a uniform temperature distribution, permit the elimination of the thermostat, and make possible a very high degree of sensitivity "   Droplets are placed on the floor and roof of the chamber and are mixed by drawing a bit of glass covered metal from one to the other with an electromagnet

Liberating known amounts of carbon dioxide within the chamber, the following calibration data were obtained

Reproducibility was also tested by measuring paramecium oxygen consumption

Table 6 shows the error to be 4 5 per cent when the total volume change is, on the average, 2 7 cmm   In table 7 the average of the probable errors,

referred to millimeter displacement per minute, is 8 per cent of the average displacement   This is large considering that average displacement per minute is 0.3 mm , and readings are made at 10 to 20 minute intervals   The chief reason for this lack of precision probably stems from the fact that the capillary is exposed to air currents and the gas may well be at different temperatures on the two sides of the index. Even in a constant temperature bath Schmitt found that temperature pockets could cause serious trouble (109)

TABLE 7

EXPERIMENT NUMBER	TRIAL NUMBER	OXYGEN CONSUMPTION		
		mm. displacement per minute		cmm. O_2 at N T.P./hr
1	1	0 30*		
	2	0 26	0 26 ± 0 04	0 66 ± 0 10
	3	0 25		
	4	0 23		
2	1	0 32*		
	2	0 35	0 33 ± 0 02	0 85 ± 0 05
3	1	0 20†		
	2	0 22	0 21 ± 0 01	0 54 ± 0 02
	3	0 22		
4	1	0 30*		
	2	0 28	0 29 ± 0 01	0 74 ± 0 02
5	1	0 14*		
	2	0 15	0 14 ± 0 01	0 35 ± 0 02
6	1	0 10†		
	2	0 08	0 09 ± 0 01	0 23 ± 0 02
7	1	0 53*		
	2	0 51	0 53 ± 0 05	1 35 ± 0 13
	3	0 58		
	4	0 50		

* Measurements made at 10 minute intervals
† Measurements made at 20 minute intervals
(From Cunningham and Kirk—J Gen Physiol 24: 135 1940.)

On the other hand the apparatus has these advantages the sensitivity can be readily altered by substituting capillaries of varying sizes, chamber volume can be readily altered, advantageously placed outlets allow for filling the chambers with various gas mixtures, two solutions may be mixed during an experiment   In a more recent paper Barth and Kirk have described a modification of this method (126a) in the direction of considerable improvement [2]

In 1941 Tyler and Berg (127) described a somewhat different capillary microrespirometer   A mercury piston was introduced to permit returning the index

[2] Footnote appears on page 75 at end of References

drop to its zero position for each reading   This device allows estimation of volume change from measurement of the amount of mercury introduced   It will be recalled that Winterstein, in 1912 (88) (89), and Duryee, in 1936 (119), described similar instruments

It is stated by Tyler and Berg that their readings are not affected by overall temperature changes, but that it is essential to avoid temperature difference on the two sides of the drop   Since this latter is far more difficult to achieve than is a constant overall temperature the net advantage is slight (109)   The instrument has the advantages that tissue being studied can be seen during an experiment (see also Stefanelli (111)) and substrate or other reagent can be added to the metabolizing material   The authors predict an error of 10 per cent for a volume change of 0 003 cmm , but state that this precision has not been attained   Experience indicates that such accuracy can be achieved with care in the selection of index drop fluid (109) (128)

Following the use of the katharometer by Shakespear (129) and by Shakespear and Daynes (130), Daynes (131) detailed the mathematical theory of the instrument   Slater (132) combined the katharometer with the capillary micro-respirometer   Oxygen consumption was measured volumetrically and carbon dioxide production katharometrically   The instrument could be used to measure an oxygen volume change of 1 cmm and a carbon dioxide volume change of 0 5 cmm   The technique has not been applied further in biology except in the macrometabolism studies of Ledig and Lyman (133) and Rabinowitch and Bozin (134)

Fenn (10) combined a conductivity cell with the differential volumeter Described in detail by Fenn (10) (100) and briefly discussed by von Ledebur (135) this method has received but little attention

Tobias and Gerard (128) further developed the capillary respirometer so that it is now convenient to follow the respiration of as many as ten or more tissue samples at once   This number can easily be expanded if necessary   Gas volume changes of the order of 0 0005 to 0 001 cmm per minute can be followed at minute to minute intervals with individual readings varying from the mean by an average of 8 per cent   Over total times of five to ten minutes the interpolated minute values have a higher precision, probably about 2 per cent

Each respirometer consists of a 90 mm length of 0 2 mm capillary tubing sealed, with de Khotinsky cement, into a 30 to 40 mm length of 1 2 to 1 5 mm thin walled tubing   The latter segment serves as tissue and reagent chamber The smaller capillary carries the index drop   After inserting tissue and absorbing reagents the open end of the chamber is sealed with plasticine   A number of units is mounted around a central heavy walled capillary extending from a glass stopper   The stopper is placed in its sheath, and the whole, submerged in a water bath, is supported in such a fashion that it can be rotated about its central axis   Thus, successive capillaries and their indices can be brought into the field of a horizontal microscope

The utilization of a very small respiration chamber (30 to 50 cmm) opposed to a large outer chamber and enclosed therein affords great stability   The chief

advantages of the technique are thus stability, high precision for very small volume changes, and the convenient multiple tube arrangement. The chief disadvantages stem from the fact that the capillaries are very fragile, and thus must be measured for each experiment and discarded. This is far too time consuming. Up to this point also it had not been possible to add substrate to the tissue during an experiment nor to observe the tissue.
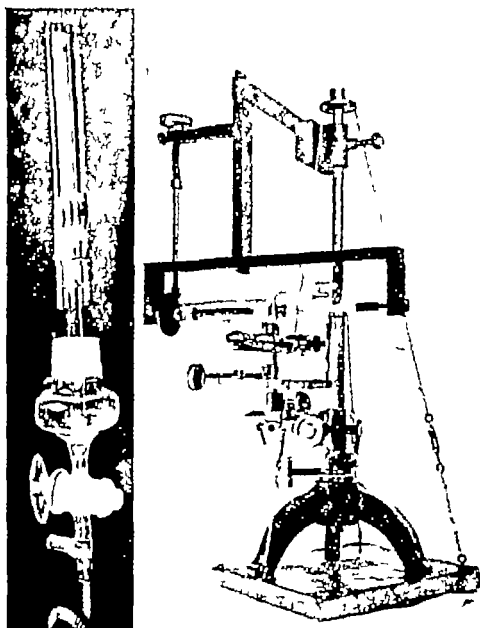
Fig. 4

In order to overcome these disadvantages the instrument has been modified. Permanent, heavy walled capillary units, expanded at one end to accommodate tissue and absorbing reagents, replace the fine capillaries. A small ground glass cap instead of plasticine closes the chamber. The units are mounted in a verti cal bank inside a brass-glass box. Brass clips into which the respirometers fit are rotatable from the outside of the box by means of a metal arm connected thereto by a packed joint. Since the tissue being studied clings to the roof of the expanded end of the unit, a droplet of substrate to be added may be placed on the floor along with a small lead shot coated with celloidin and paraffin

Rotation of a single capillary at any time then causes the mixing ball to drag the reagent around to the tissue   Greasing of the cap-respirometer joint makes it transparent and thus allows visual observation of the tissue being studied

In 1942 Scholander described (138a, 138b) a volumetric respirometer sensitive to about 0 01 cmm per hour   It embodies principles similar to those utilized by Duryee and by Tyler and Berg

Miscellaneous techniques   From time to time micromethods have been developed for gas analyses based on the principles of selective absorption of gases by specific reagents (136) (137) (138) (139) (140) (141) (142)   Perhaps the most noteworthy applications to biological problems were those of Krogh who, following the lead of Timiriazeff (143), developed the tonometric method (94) (144) (145) to a high degree of sensitivity and precision (146) (147)

The technique used by Hill (148) in a study of rubber permeability has not been otherwise applied   It involved the detection of oxygen by luminous bacteria which can be seen to glow when oxygen partial pressure is as low as 0 0053 mm Hg   Other such data on minimal required oxygen concentration have been obtained (149) (150)

Dublin, Boothby, Brown and Williams (151) analyzed mixtures of He, O_2 and N_2 by determining sound velocity in the mixture   The method has no qualitative value   No effort has been made to adapt it to micro-studies, and it is unlikely that such adaptation will ever be made because of limited sensitivity

McAlister (152) developed a spectrophotometric technique for the study of plant respiration and photosynthesis (153) (154)   In 1939 Pfund (155) modified the method by the introduction of a phonic interrupter wheel and pitch detector   Simple calculations show that an adaptation of the method to micro levels, of the order discussed in this paper, would involve very serious technical difficulties   No effort has been made at such an adaptation

Interferometry has been applied to problems in metabolism (156) (157) (158) The great cost of the apparatus and its relative complexity have overshadowed the assets of great precision, speed and sensitivity

Utilizing the interferometer principle (159) a sensitive indicator of pressure change has recently been developed by the author   To the surface of a microscope slide there is fastened, with shellac, a cover slip (about 0 1 mm thick) through which has been drilled a hole (convenient size for preliminary experiments has been about 2 mm diam)   Over the hole a thin organic film is spread and is held in place by another, similarly drilled, cover slip and shellac Multiple reflections of monochromatic light in the gap between the cover slip and the microscope slide produce circular, concentric interference fringes   As pressure change causes the membrane to be displaced with respect to the fixed slide the fringes are seen to shift   Because of the extreme thinness of the film the instrument is very sensitive   (Neither the film nor the slide need be silvered or aluminized   Reflectivity is high enough to produce bright, clear fringes) The instrument responds to pressure changes of the order of 0 05 mm water (about 0 004 mm Hg)

Summary of sensitivities Recently several authors (57) (160) have published lists of so-called sensitivities for the various microgasometric methods These have expressed sensitivities in terms of cubic millimeter volume change measured by a given shift of meniscus position, in the case of the manometric methods Such lists assign different sensitivities to different constructions of capillary respirometers incorporating capillaries of different dimensions and utilizing different methods for reading meniscus position Obviously these distinctions are arbitrary

Table 8 gives the limits to which the sensitivities of the various genera of apparatus have so far been pushed Several of them are capable of development to considerably greater sensitivity Errors estimated are those which might reasonably be expected were the indicated volume change measured only once

TABLE 8

INSTRUMENT	MEASURES	SMALLEST MEASURABLE VOLUME CHANGE— ESTIMATED DIRECTLY FROM TWO READINGS	INTERPOLATED FROM SERIES OF MEASUREMENTS	ERROR OF METHOD AS DESCRIBED IN TEXT
Biometer	CO_2	2.5×10^{-2} cmm		6%
Winkler titration	Dissolved O_2	28 cmm		1–2 5%
Indicator	Dissolved CO_2	5×10^{-1} cmm		10%
Optical lever	CO_2 O_2 etc	1 cmm /hour		2–3%
Gradient tube	Volume change in liquid systems	3×10^{-7} cmm		A few %
Cartesian diver	CO_2 O_2 etc	$1–2 \times 10^{-2}$ cmm		50–100%
		5×10^{-3} cmm		A few %
			$1–2 \times 10^{-3}$ cmm	A few %
Polarograph	Dissolved O_2	1×10^{-2} cmm		Very precise
Microcapillary	CO_2 O_2 etc	5×10^{-4} cmm		8%
			5×10^{-4} cmm	A few %

Reactions suitable for the calibration of various microrespirometers Numerous reactions, proceeding at a known rate, have been utilized for the calibration of micromanometric systems of various sorts Some of these, such as the liberation of carbon dioxide from bicarbonate by excess of acid, and the fermentative processes need no additional mention Some other suitable reactions are noted below with references Not all have been used for the purpose indicated

Oxidation of cysteine

Harrison Biochem J 18 1009, 1924
Boell Needham and Rogers Proc Roy Soc London (B) 127 322 1939
Kharasch Legault Wilder and Gerard J Biol Chem 113 537, 1936

Decomposition of oxaloacetic acid

Ostern Hoppe Seyler s Ztschr 218 160 1933
Boell Needham and Rogers as above

Decomposition of acetoacetic acid

Ostern, as above
Boell, Needham and Rogers, as above
Ljunggren   Ber d deutsch Chem Gesell **56** 2469, 1923
Ljunggren Biochem Ztschr **145** 422, 1924
von Euler u Olander   Ztschr anorg Chem **147** 295, 1925
Burki und Schaaf Helv Chim Acta **4** 418, 1921
Widmark Acta med Scand **53** 393, 1920

Decomposition of alpha-alpha dimethylacetoacetic acid

Pedersen   J Am Chem Soc **51** 2098, 1929

Decomposition of nitramide

Bronsted und Pedersen   Ztschr physik Chem **108** 185, 1924

Decomposition of nitroso-triacetone-amine

Bronsted and King   J Am Chem Soc **47** 2523, 1925
Francis and Gliddens   J Chem Soc **101** 2358, 1912
Francis and Geake   J Chem Soc **103** 1722, 1913
Francis, Geake and Roche   J Chem Soc **107** 1651, 1915

Peroxide decomposition by catalase

Heatley, Berenblum and Chain   Biochem J **33** 53, 1939

Cathodal reduction of oxygen

Davies and Brink   Proc Am Physiol Soc 69, 1941

Estimation of amount of metabolizing material   Since, for purposes of comparison, it is necessary to refer respiration intensity to the amount of respiring material present, the accurate estimation of the latter becomes an important factor in determining the significance of such data   The problem is equally difficult in macro and micro studies from the point of view of partitioning respiration intensity amongst the various cellular constituents present in a bit of tissue   As tissue homogeneity increases, this matter becomes progressively less troublesome   In micro-studies there is the more primitive problem of simply determining the total amount of material present

The commonest method for getting at the amount of respiring material involves the determination of dry weight   The ordinary Kuhlman microbalance can be used for dry weights of 80 μg or more (44)   With smaller amounts of tissue serious error can be introduced   For weights of less than 80 μg the Donau microbalance (44) (161) is recommended   Torsion balances sensitive to 0 002 mgm are commercially available   For weights of the order of 0 0001 mgm the quartz fiber balance should be used   In 1941 Lowry described such a balance with a sensitivity of about 0 03 γ, and with reproducibility of 0 1 γ The maximum load capacity was about 250 γ A modified type of quartz fiber balance has also been described by Bazzoni (169) (170)

Total nitrogen determination can be done accurately on small amounts of tissue, and nitrogen content can be used as the standard of reference   Like the

measurement of dry weight, this method does not differentiate between respiring and non respiring material   Referring respiration intensity to dry weight may also give a false picture of the actual situation since various organs and tissues and parts of organs have quite different water contents

Nucleic acid determination may be used to differentiate cellular from non cellular material (44)   After suitable extractions nucleic acid phosphorus may be determined by the method of Berenblum and Chain (162)

Recently Caspersson (163) has developed an ultraviolet absorption method for the determination of nucleic acid compounds which have a characteristic absorption curve in the ultraviolet spectrum   The curve can be gotten on microscopic sections or smears   Its shape identifies the compound, and the height of the curve can be used as a measure of the amount present in the field measured   This method has not, to date, been used in conjunction with respira tion studies, though it has been used to follow the intracellular metabolism of nucleic acid (164)

Cell counts are, of course, ideally suited to the study of cell suspensions Recently the use of counting techniques has received a certain impetus in the study of solid masses of tissue also   Glick, Holter, Linderström Lang and Seberg Ohlsen (165) developed a cell counting technique in connection with studies on enzymatic histochemistry   The method consists of cutting frozen sections from a core of tissue using alternate sections for histological and enzyme analysis The authors point out that counting nuclei is much preferable to counting cell outlines   It may be noted that when nucleoli are present as single and constant structures they would afford a still better index of number of cells (166) (167)

For muscle, Grossman (168) has found that maceration in 5 per cent citric acid causes extrusion of nuclei   These might then be taken up, homogenized by shaking and counted in a hemocytometer chamber   Recently Pearce and Gerard (116) have macerated tissue by grinding with a small glass pestle in con centrated acetic acid   After maceration the tissue is stained, shaken in a blood pipette, and nuclei are counted in a hemocytometer chamber

The measurement of tissue volume has also been attempted (128)   Tissue is forced into a capillary of known diameter and the length of the cylinder it occupies is then measured directly   Adequate data for evaluation are not yet available

The improvement in microgasometric techniques has gone far enough so that use of bits of tissue sufficiently small to allow direct counting of all cells present is feasible (166)   Thus, in contrast to the work of Glick et al , cell counts can be made directly on the sample which has been studied metabolically   This would also allow for measurements of cell and nuclear dimensions   Such work is, at present, in progress in this laboratory

In conclusion, it is important to emphasize that none of these methods affords a basis for the apportionment of total respiration intensity amongst the various cell types present in a given piece of tissue   The importance of knowing something of this distribution is great and the problem presents a very real challenge to workers in the field

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[1] Since this review has gone to press Cunningham and Kirk (Jour Cell and Comp Physiol 20 119 1942) have published a paper describing a capillary respirometer sensitive to 5×10^{-5} cmm The respirometer is of the open type and represents a development of the Kalmus technique The criticisms levelled at the Kalmus method have been circumvented

INTERRELATIONS OF CALCIUM AND ASCORBIC ACID TO CELL SURFACES AND INTERCELLULAR SUBSTANCES AND TO PHYSIOLOGICAL ACTION

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A better understanding of the physiological effects of vitamins in plants and in animals is a goal toward which a number of different lines of investigation are now being directed   Some insight has been gained concerning the mode of action of several of these substances in animals but comparatively little is known of their functions in plants   It has been demonstrated that several members of the vitamin B complex participate in respiratory processes and because of its reducing action, it has been suggested that ascorbic acid also is concerned in respiration   There is as yet, however, no considerable amount of evidence clearly indicating such a relationship in intact tissues   Ascorbic acid is known to be involved in the synthesis of collagen and reticulin, carbohydrate-containing sclero-proteins found in bones and connective tissue and in smaller amounts in other animal tissues   These frame-work proteins all presumably contain small amounts of carbohydrates and function in animals in much the same way as do the anhydrides of hexoses and pentoses in the cellulose, lignin, pentosans, etc , in plants   It has not been discovered whether ascorbic acid is concerned primarily in the activities occurring within cells or in the synthesis of essential substances outside of cell boundaries   An impressive amount of evidence has been accumulated which suggests that it is concerned in some way with determining the retention and absorption of water   Whatever its mode of action may be, there is considerable evidence that calcium is involved in its functioning

The following review of literature and discussion represent an endeavor to correlate some of the known but hitherto widely scattered facts concerning the physiological interrelations of ascorbic acid and calcium in animals and plants and to point out suggestive similarities in their action in the two groups of organisms   The subject is discussed under the following topics

    I Relation of calcium to the boundary structure of cells

   II Ascorbic acid in relation to cytoplasm and cell surfaces, cell walls, and intercellular substances

  III Some aspects of the chemical nature of the cell wall and intercellular substances

  IV Loss of ascorbic acid in metabolic processes

   V Similarity of some physiological effects of ascorbic acid and calcium

I RELATION OF CALCIUM TO THE BOUNDARY STRUCTURE OF CELLS   1 *In animals*   The protoplasmic cell surface or plasma membrane is generally considered to be a delicate but essential structure, not many molecules in thickness and containing lipid, probably associated with protein, as a major component   Microscopic studies have revealed the existence of a readily visible

cortical layer of cytoplasm, the behavior of which has been shown by Heilbrunn and co-workers (58) to be largely controlled by calcium (see also Just (67)) These investigators observed that calcium ions have a stiffening effect upon the ectoplasm of amoeba but cause a decrease in viscosity of the endoplasm It has also been found that calcium in the surrounding medium is not essential for the formation and maintenance of the surface layer   Chambers (23, 24) reported that Arbacia eggs immersed in a mixture of sodium and potassium chloride in proportions of 19 1, in the concentrations found in sea water, and at a pH of 7 0 will divide for many generations, the blastomeres separating as individual cells because of lack of intercellular cement material   He concluded that an addition of surface material from the underlying cytoplasm must occur If the surface layer is injured at some point, the cytoplasm flows out and disintegration of the surface layer spreads out over the entire cell   However, repair of an injury, which exposes the internal cytoplasm, is impossible in the absence of calcium because of inability of the protoplasm to form a new interface In the presence of a low concentration of calcium, partial coagulation around the point of injury occurs and a new protoplasmic surface layer develops at the boundary between the healthy cytoplasm and the coagulating surface   The latter is supposed to afford a solid substrate against which surface substances may accumulate   The repair of the protoplasmic surface thus depends upon a previous formation of an extraneous coat.

As cells become older the surface may become modified by secretions of various types such as mineral matter in the formation of bone   Such surface coatings are not essential to single cells but they are necessary for multicellular organisms This is true, for example, of the hyaline layer in developing eggs, a material which Kopac (73) considered to function as an intercellular cement   Previous observations by Galtsoff (40) and Gray (47) had suggested that the surface layer of isolated cells is homologous to the intercellular cement of multicellular organisms   Intercellular substances form the foundation of all fibrous structures such as the bone matrix, cartilage, dentin, the vascular endothelium, and of all non-epithelial cement substances   In some tissues such as cartilage and connective tissue the volume of intercellular substances may be very large in comparison to that of the cell itself   In the absence of calcium the intercellular cement breaks down to a soluble substance, thereby becoming dispersed in the medium   Many investigators have contributed to our knowledge of calcium as a stabilizer of this structure in animal tissues (117, 118, 119, 59, 79, 47, 57, 58, 98, 25, 23, 73, 152)

Zweifach (152) found that the permeability of the capillaries is determined almost entirely by the nature of the cement, and apparently also in part by an adsorbed layer of protein, rather than by the cells themselves   He observed that lack of calcium in the perfusion fluid weakens the cement substances between the endothelial cells with the result that extravasation of both fluids and blood cells occurs   When the circulating fluid is changed to one containing calcium the cement substance is re formed and the normal permeability is re established   Zweifach suggests that a similar cement probably exists in other epithelial membranes such as periosteum, kidney tubules and epithelium

There is comparatively little known concerning the ratio of calcium in the interior of the cell to that in surface membranes and surrounding fluids   Scott (130) found that practically all of the calcium in the nerve fibers is located in the nerve sheath   He concludes that since only a small portion of the sheath contains aqueous channels, the local concentration may be ten to twenty times that which would be expected if the calcium were uniformly distributed in the nerve fiber   The distribution of calcium between the cells and extracellular fluids of skeletal muscles and liver in dogs was studied by Eichelberger, McLean and Catterall (32)   They applied accepted methods for calculating the distribution of sodium and potassium to similar calculations for the distribution of calcium and magnesium   Their results indicate that the concentration of calcium in the cells is 80 per cent lower than that in the extracellular fluid   They considered the possibility that all the calcium in the tissues may be extracellular and concluded that unless some of it is actually within the cells, a part of it must be in a unionized combination with some extracellular substance other than the protein of the extracellular fluid, possibly the connective tissue fibers They did not suggest, however, the possibility of a linkage of a portion of the calcium with a constituent of an intercellular cement substance to form an unreadily soluble compound

There is considerable evidence which shows that the stability of intercellular substances is attained only when bases in addition to calcium are present in the surrounding medium (99)   The generally accepted view is that magnesium, potassium and sodium all enter into the final equilibrium in the animal cell and that one cannot completely compensate for the absence of another (47, 48)

2 *In plants*   The plasma membrane of the plant cell as in the animal is supported by a "gel" layer of cytoplasm (126) but there is still insufficient evidence to support the assumption that calcium is essential to its formation Comparatively small amounts of substances are found between the cells in plants   The relationship of the middle lamella to the surrounding cells is clearly shown and it is distinctly a double layer deposited by the two adjoining cell surfaces (1)   In a limited sense the wall of the plant cell, especially its outer older portion, is comparable to the intercellular substances in animal tissues By special staining it can, in fact, be shown that at least in some types of tissues, the intercellular material between adjoining cells is, like the middle lamella in plant tissues, really a double layer

Reed (109) found that nuclear division occurs in spirogyra in the absence of calcium but cell division often is not completed, because of lack of formation of the middle lamella, which is known to consist chiefly of calcium pectate (105, 90, 91, 52, and others)   True and co-workers (140, 141) have made important contributions to our knowledge of the rôle of calcium in cell walls   Eckerson, working in co-operation with these investigators, employed microchemical methods (141) in a study of seedlings grown in solutions of potassium salts similar to those from which True and Bartlett (142) had previously found a leaching of ions   She observed "(1) that ions readily entered the cells of the roots, (2) that within twenty-four hours calcium ions began to diffuse out of the calcium pectate of the middle lamella, (3) potassium pectate was formed instead

of the calcium salt and this substance being readily soluble in water soon dissolved, (4) at this stage, sugars amino acids and salts, chiefly magnesium, diffused rapidly out of the roots " In similar tests with magnesium solutions it was found that magnesium pectate replaced calcium pectate in the cell walls These studies showed that the action of potassium and magnesium in the surrounding solution upon the calcium pectate of the middle lamella not only changed the character of the wall but also altered greatly the permeability of the protoplasmic membrane next to it   That calcium is necessary also for the normal development and functioning of the cytoplasm is recognized (141 136, 11)   It should be emphasized, however, that the evidence presented above indicates clearly that calcium has a function in the nutrient medium aside from its uses within the cells

In conclusion, it may be stated that calcium functions in the extraneous coats of cells in both plant and animal organisms   In its absence, these coverings are dissolved, the constituent substances becoming dispersed in the surrounding medium   There is considerable evidence also which indicates that calcium is essential for the production and maintenance of the cytoplasm   It increases the viscosity of the cytoplasm in the cortical region and decreases that in the interior of the cell

II ASCORBIC ACID IN RELATION TO CYTOPLASM AND CELL SURFACES, CELL WALLS AND INTERCELLULAR SUBSTANCES   1 *In animals*   One group of investigators hold that ascorbic acid is essential for the production of the intercellular matrix, a structure consisting of collagen or a related substance as a basic constituent whereas another group maintains that its primary influence is upon the functioning of cells   The former group (3, 151, and others) claim that if ascorbic acid is unavailable a liquid product which lacks the ability to gel is formed in the intercellular areas   In a study of scorbutic tissues Wolbach (150) observed vacuolation of the fibroblasts, usually in their extremities, and held that the intercellular liquid very probably has its source in the cytoplasmic vacuoles   He thought that the vacuoles may be considered as evidence of degeneration (cytoplasmic presumably) or the consequence of secretion of an abnormal product   He accepted the latter interpretation   Dalldorf (30) who also holds this view describes the action of ascorbic acid as follows

Under certain conditions the type cell the fibroblast lies in an amorphous ground substance within which fibrils (reticulum) are formed which may in turn become gathered into wavy bands of collagen   In this transformation the fibrils seem to become cemented together by a translucent matrix the formation suggesting a colloid phenomenon the setting of a gel   It is precisely this phase of the formation of intercellular materials which may be completely controlled by vitamin C   Thus in guinea pigs which have been depleted of vitamin C the ground substance and fibroblasts are present as in health but fibrils or collagen are not formed   When the deficiency is satisfied translucent bundles or masses of collagenous materials reappear within eighteen hours   The formation of intercellular material of bone (osteoid tissue) and of teeth (dentin) may be similarly controlled by with holding or supplying vitamin C

Höjer (64) advanced the hypothesis that vitamin C (ascorbic acid) is necessary for the proper functioning of cells in general and for specialized cells in particular

Fish and Harris (38) and Ham and Elliott (50) also accepted the view that vitamin C is somehow concerned in the metabolic processes of cells   The latter investigators held the evidence for the gelation theory to be entirely inadequate   MacLean, Sheppard and McHenry (84) also obtained no evidence in support of the gelation theory   They showed that ascorbic acid deficiency causes a failure in those special cells which are concerned in the formation of calcific tissue, as in bones and teeth and the intercellular cement   Morphological changes in the blood vessel walls have not been observed in ascorbic acid-deficient animals but presumably the intercellular cement breaks down as it does in the absence of calcium   Meyer (96) presented histological evidence of effects of lack of ascorbic acid upon the structure of the cells themselves   He observed a widely distributed depletion and destruction of the cytoplasm and cell membranes in guinea pigs kept on a scurvy-producing diet   Vacuolation was common in many organs   Most of the other workers in this field have not observed a degenerating condition of the cytoplasm and cell membranes but its occurrence should not be surprising in a disease which, in severe form, causes such general tissue breakdown   The lack of agreement as to the range of tissues which may show histological defects in scurvy is probably in part a consequence of differences in the severity of the disease in the animals examined

In recovery from scorbutus, the collagen deposit is said by Wolbach (150) to be restricted to the near vicinity of the fibroblasts, the fibrils tending to follow the cell outlines   He described the first material with the staining properties of collagen as homogeneous, resembling lightly-stained amyloid and closely applied to the cytoplasm of the cell itself and in some instances to fibrils extending beyond the apparent outlines of the cells   He noted that it was also present about cells when no fibrils were observed   Hansen (51) and Mall (87, 88) held that collagen fibrils originate as a direct modification of the surface cytoplasm of the fibroblast and its processes   Lewis (77) presented evidence supporting this view   She observed fibrils in an early stage as slightly more refractive lines within the cytoplasm of the cell which later formed slender fibers grouped in bundles outside of the cell   Other investigators (6, 7, 8, 54) have described collagen fibrils as forming in collagen independently of the immediate proximity of cells   It is possible that certain phases of collagen synthesis occur within the boundary of the cell but that completion of the product is intercellular   Hence, although histological evidence may suggest that the action of ascorbic acid is primarily intercellular, to designate it as such without regard to the less readily observable intracellular phases seems unjustifiable

If ascorbic acid really is concerned in extra-cellular processes its concentration at cell surfaces might be expected   There is some evidence from cytological studies, which, though inconclusive, because the reaction employed is not specific for ascorbic acid, nevertheless shows that a silver nitrate-reducing substance is present in the cytoplasm and intercellular spaces (20, 21, 147)   Bourne called attention to the possibility that different lipoids may be concerned at times with the silver reaction in the adrenals

Evidence of an effect of ascorbic acid upon surface activity of colloidal solu-

tions is shown in work reported by Keller and Künzel (69), "surface activity" being understood as the force which causes dropping fluids to assume a spherical form, the smaller the drops the higher the activity. All substances which alter the surface tension of a solution medium, such as water, are said to be surface active. The investigators found that solutions of thiamine (vitamin B_1), riboflavin (vitamin B_2) and ascorbic acid do not show surface activity in themselves, but that under certain conditions they can influence this type of behavior in lecithin-cholesterol mixtures. The greatest activity was observed in the higher dilutions and also under the closest approach to physiological conditions with respect to pH. It was pointed out that the physical effects of vitamins may be very important and that their mode of operation cannot be explained by chemical structure and affinity alone.

2. *In plants.* Reid (112) made a study of some of the physical and chemical changes occurring in the growing region of the primary root of cowpea seedlings. The following regions were examined: embryonic, intermediate to embryonic

TABLE 1

Relative values per cell in cells of different ages

REGION	GREEN WEIGHT	DRY WEIGHT	WATER	NITROGEN	PHOSPHORUS	SURFACE AREA	TOTAL ASCORBIC ACID	TOTAL ASCORBIC ACID/TOTAL SURFACE AREA
Embryonic	1 0	1 0	1 0	1 0	1 0	1 0	1 0	1 0
Intermediate	2 8	2 0	3 5	2 0	1 8	2 3	2 6	1 1
Elongation	13 7	4 8	16 9	4 0	4 2	7 3	7 9	1 1
Intermediate	32 0	8 0	43 2	5 8	5 3	11 8	11 8	1 0
Maturation	32 0	7 0	46 3	5 5	4 5		11 3	

and elongation, elongation, intermediate to elongation and maturation, and maturation. Continuous increases in volume, water, dry matter, nitrogen, and phosphorus per cell occurred during the transition from the embryonic to the mature condition. The results of these studies (112, 113) expressed in terms of relative values per cell (the average value for a cell in the embryonic region being taken as 1) are summarized in table 1.

It may be observed that the increase in surface of expanding cells parallels the increase in ascorbic acid and that surface area increase is the only one of the various factors studied which shows a parallel relationship with increase in ascorbic acid. Phosphorus gained relatively as rapidly as nitrogen and possibly slightly more rapidly during the time of most active expansion and it was suggested that an accumulation of a phospholipin such as lecithin or a closely related compound may be involved in this increase. Bessey, Menten and King (18) reported a close relationship between ascorbic acid and complex lipids in animal tissues. In a study of the distribution of ascorbic acid in animal tissues, Bourne (21) pointed out that cells with a high concentration of substances such as lipochrome pigments, lipoids and fats, cholesterol and its esters contain large

quantities of ascorbic acid   The increase in phosphorus during the embryonic and early growth phases of the root cells above mentioned and the decrease during maturation are presumably related chiefly to changes in the content of nucleoproteins and nucleic acids   The relatively high content of ascorbic acid per unit of dry matter in the region of expansion was an outstanding feature of the observations   The dry substance in the region of elongation contained 60 per cent more ascorbic acid than that of the embryonic region where nuclear activity is high and 30 per cent more than the region of maturation where cellulose accumulation appears to be the dominant activity   The data suggest that the increase in ascorbic acid is associated with an increase in the cytoplasm or some of its constituents

Further investigations of similar nature with other types of plants are necessary before drawing conclusions as to the significance of the results cited above The quantitative relations with surface area here found do suggest, however, that ascorbic acid may tend to be concentrated at and near cell surfaces in metabolically active cells and that it may consequently have important functions in connection with cell expansion, development of the wall, and absorptive and secretory processes   A discussion of suggested relations of ascorbic acid to these activities will be presented in section V   Additional support for the hypothesis that ascorbic acid functions at cell surfaces is afforded by evidence suggesting that the surfaces of plant cells contain substances with an acid reaction (102, 103)   Howe (66) found that the pH values of root hairs range from 6 0 to 6 8 in the plants tested, but she did not state whether the acidity could be ascribed in part to the presence of pectic materials known to be important constituents of root hair walls (120)   In investigations made with a wide variety of plant materials the writer has observed that ascorbic acid leaches out readily from most of them when coarsely comminuted in metaphosphoric acid and the mixture allowed to stand for a half-hour or longer   These observations suggest that the ascorbic acid in the tissues, with the possible exception of that in the embryonic cells, moves readily from cell to cell through or on the surfaces of the cell walls   Another type of observation which is of interest in connection with a cell-surface relation of ascorbic acid in absorption was made in tests with highly illuminated, actively growing cowpea plants (Reid, unpublished data), the roots of which were immersed in black-walled jars containing a dilute solution of indophenol (1 125,000)   A reduction of the dye equivalent to a milligram of ascorbic acid per plant occurred within a period of two hours This reducing activity was equal to about one-ninth of the total reducing activity in the minced and extracted tissues of similar plants with all organs included In other tests, following decolorization of the solution, additional dye was added, this procedure being repeated throughout the day   The total quantity of dye then reduced in the solution was equal to approximately one-half of the calculated total indophenol-reducing activity within the plant   The substance oxidized by the dye was apparently again reduced in the tissues, as determinations of the reducing activity of plants kept in the indophenol solution showed that no appreciable diminution had occurred during the period of immersion of

the roots   When plants in the bleached dye solutions were subsequently kept in darkness for several hours a partial return of color in the solution was noted, but a change to red color indicated a more acid condition of the medium   A marked turbidity of the external solution signifying a leaching of substances from the roots was observed following the dye treatment, the leaching being also noticeable after a period in darkness   These tests should be repeated and other tests made with plants kept in darkness during the period of immersion of the roots in the dye   Preliminary studies have shown little reducing activity   It thus appears that the action of the dye on the roots occurs chiefly as a consequence of exposure of the tops to light   The nature of the reaction of the dye with the root cells is not clear, but, because of its rapidity and the rather large size of the dye molecules, it may presumably be related to the existence of a readily oxidizable substance in the membrane (58, 93, see also 22)   Further experiments with the dye solution adjusted at different pH values should give somewhat more definite clues as to whether or not ascorbic acid is primarily involved in the reaction

Schreiner and Sullivan (128, 129) studied the reducing action of the roots of wheat seedlings on solutions of sodium selenite and found that it was stimulated by a faintly acid reaction and by light and was most marked intracellularly in the parenchyma cells of the root tip   They considered it probable that the reduction is due to the metabolic activities of the roots, the reaction involving some unstable non-enzymatic substance comparable to the oxyorganic acids, or to complex, unsaturated compounds comparable to dextrose and levulose or unsaturated fatty acids   (It is now known that ascorbic acid will reduce solutions of sodium selenite)   Heffter (56) concluded that a type of reduction process, apparently similar to that studied later by Schreiner and Sullivan, is caused by the labile hydrogen of the sulphydryl group of certain proteins   It should be possible by further investigation, using selenite solutions with different hydrogen ion concentrations, to determine whether the type of reduction in intact roots herein described may be concerned primarily with ascorbic acid or with sulphydryl compounds.

III Some aspects of the chemical nature of cell membranes cell walls and intercellular substances 1 *In animals* The physical state of intercellular materials has been studied by a number of investigators and as a result of their observations the viewpoint has developed that "cells repose in a jelly, not upon a water bed" (27, 28 17, 85, see also Editorial, The Lancet (74))   On the other hand there is comparatively little definite knowledge concerning the chemical nature of the intercellular substance in animal cells   Gray (47) stated that in the connective tissue of young vertebrates it appears to consist of mucoid (a glyco-protein known to contain sugars and uronic acids) with fibrils of collagen and elastin   Halliburton (49), Chittenden and Gies (26), and Van Lier (143), also have presented evidence of the occurrence of mucoids between the fibrils of connective tissue   Data obtained by Bensley (17) in a study of the reaction of paramoecin in bullae of subcutaneous connective tissue suggested the presence in the intercellular areas of a viscid substance, possibly acid in reac

tion, digestible by pancreatin but not by pepsin, and with staining properties resembling those of mucins   The fact that this substance could be extracted with lime water also suggested that it may be mucoid   Zweifach (152) found that the intercellular cement substance of the endothelium of the blood vessels behaves as though it were a calcium salt, probably a proteinate, and that it is digestible by trypsin   The possibility of a carbohydrate linkage with the protein was not suggested   Ferry (37) studied the jelly layer of Arenicola eggs and concluded that it is essentially a polysaccharide

A possible relation between the water-holding capacity of tissues and their content of certain types of carbohydrate-protein complexes is suggested in studies conducted by Heringa and Weidinger (61)   They measured the amount of water taken up by the cornea and sclera after they were in equilibrium with water vapor of a certain partial pressure and found that in water vapor tension above 0 9 (90 per cent) the sclera fails to take up additional water whereas the cornea continues to swell up to about 1000 per cent of its dry material   They interpret this result as seeming to indicate that there is inserted between the connective tissue fibrils a substance, probably mucoid, which differs in cornea and sclera Their hypothesis is based upon the fact that the cornea was found to contain 20 per cent of mucoid and the sclera 13 per cent and that after the extraction of the mucoid the cornea lost the greater part of its swelling capacity   Using the Sorensen-Haugaard adaptation of the Orcin reaction, Grassman and Schleich (46) studied the carbohydrate content of animal skin and found that it contained unimolecular proportions of glucose and galactose   From this finding they concluded that the carbohydrate was present in the form of lactose   They also attempted to determine the significance of the substance surrounding the collagen fibers in a study of collagen fibers from cow's skin   They consider that their results afford good reason for the assumption that the lactose is a constituent of collagen and not of the chemically undefined interfibrillar substance   However, their evidence that the interfibrillar substance does not also contain small amounts of carbohydrates is not entirely convincing   Schneider (127) also has shown that collagen contains a small amount of firmly bound sugar   Beek (15) found that the carbohydrates from collagen were not fermented by galactose-active yeast and concluded that neither d-glucose nor d-galactose forms a considerable part of the sugars   He expressed the opinion that the carbohydrates may be l-isomers of the two sugars and noted that Bell and Baldwin (16) had found l-galactose as a component of a polysaccharide of animal origin

Bensley (17) suggested that the cement substance of collagenic fibers may be a modified form of the ground substance and that the chemical and physical differences between reticular and collagenic tissue may be due to this difference in the relation of the fibers to the ground substance   She says that "the difference in reactions of reticular and collagenic fibers with dilute acetic acid may be explained on the basis of this relation   Undoubtedly the ground substance is in a complex colloidal state   It is well known that weak acids increase the capacity of lyophyllic colloids to absorb water   The hydration of the ground

or cement substance due to the acetic acid may render its refractive index the same as that of the fibrillae of the collagenic fiber and thereby make them both invisible   That reticular fibers do not react in this way may be explained by the fact that the ground substance enveloping them is not in the nature of a cement substance, i e , there is not so much of it and it is not so condensed   Therefore, its hydration capacity is not increased to such a great extent and the refractive index is not appreciably changed "   Observations made by Heringa and Weidinger (62) suggest that differences in chemical composition of reticulin and collagen are, in part at least, responsible for the differences in behavior   They also found that reticulin does not swell appreciably in acids and that it has a higher sulphur content than collagen   The sulphur, chiefly present as cystine, is in side chains, and by tending to diminish the side chain distances tends to prevent the intermicellar penetration of water   Thus in a very early stage of development a non-swelling substance, reticulin, is contained in skin, thereby enabling it to exist in a watery medium   Later, this substance changes to collagen which allows water to pass   It seems probable that during maturation of tissues changes may occur also in the ground substance as suggested by Bensley   Further study of this aspect of the problem appears necessary

Northrup and Kunitz (101) accounted for the high viscosity of gelatin and its variation with pH by the existence of two forms—one form occurring as insoluble micellae and the other a soluble form distributed between the micellae and the outer solution   They do not say whether or not it may be possible that differences in solubility of fibrillar and interfibrillar substances are involved in producing these results

Höjer (64) reported finding a collagen atrophy in the cartilage in scurvy, especially marked in the columns of the proliferating zone and in their neighborhood   He observed that sections stained with methylene blue did not differ from the normal in regard to chondroitin-sulphuric acid   It would appear from this observation that if ascorbic acid is used directly in the production of a protein-carbohydrate complex it must be in relation to some special type which presumably is used in comparatively small amounts such as in the intercellular cement of the epithelial cells of certain tissues

2 *In plants*   The chemical nature of the outer wall layer or middle lamella of young expanding cells is not known definitely   In the early stages it is generally considered to be chiefly pectic material such as protopectin or pectin which later changes into insoluble compounds mainly as the calcium salt of some pectic substance   There is lack of agreement as to the exact form of the latter though many workers have considered it to be calcium pectate, a salt of pectic acid   It is not known whether or not these substances are linked to protein or more probably to phosphoprotein molecules in the early stages of cell surface formation   After and also during the later phases of cell expansion, the pectic material is generally supposed to combine with calcium to form the insoluble compound above mentioned   Pectic acid is known to be a complex molecule composed of sugars such as glucose or galactose and in some cases pentoses, together with uronic acids, chiefly galacturonic and glycuronic   When ascorbic acid is heated

with hydrochloric acid, a carbon atom is detached and the remaining compound gives the furfural reaction characteristic of pentoses

Farr and Eckerson (36) observed that cells of higher plants (see also, Barrows (12)) are surrounded by wall-layers composed of cellulose particles embedded in an isotropic matrix, which was shown by later work (53, 35) to have some of the characteristics of pectic material   Wergin (146) studied the structure of cotton fibers and reported also that the fibrils consist of cellulose particles, considerably smaller than the units described by Farr and Eckerson   Wieler (149) investigated the cell-wall structure in a large number of plants including that of cotton fibers and he, too, observed cellulose particles not very different in size from those described by Farr and Eckerson   Concerning the nature of the cementing material surrounding the cellulose particles Farr (35) states that there seems to be no reason for considering it as anything other than a surface coating left by the cytoplasm upon each separate particle and between the successive layers of fibrils which form the wall lamellae   (Chambers (24) has pointed out'that in animal cells the outer hyaline layer is formed as a secretory product left behind by the receding protoplasm)   It is to be inferred from the general trend of evidence in the studies of Farr and her associates that the cytoplasm, perhaps especially the cortical layers, contains appreciable amounts of pectic materials Compton (29) has shown that the high hygroscopic moisture content of cotton fibers at various stages of development is due chiefly to the presence of water-soluble carbohydrates and pectic material

There is some indication of chemical similarity in the cementing material which binds together the cellulose layers in the plant cell wall and the collagen and elastin fibrils in the intercellular matrix of animal tissues, namely, the presence of complex carbohydrates containing sugars and in many cases also of uronic acids   If present in both kinds of cells the function of these substances is likely to be somewhat similar in plants and in animals and they may play highly important rôles as hydrating and geling agents   The results of Heringa and Weidinger (61, 62) previously mentioned strongly suggest the importance of these complexes in determining the water-binding capacity of the connective tissues in animals

Ruskin (123) has shown that ascorbic acid combines readily with calcium and Ruskin and Jonnard (124) that it combines with protein but much more readily if calcium also is present   The possibility of a relation of ascorbic acid to the pectic-like substance which may be involved in gelation processes in plant and animal tissues brings to attention a crucial problem   Available experimental evidence thus indicates that ascorbic acid may be qualified to act as a carrier of calcium in embryonic tissues but it is possible also that it helps to prevent premature calcification, and that it may be utilized directly (though possibly only in plants) in the synthesis of some special carbohydrate fraction   The quantitative relations which have been found between ascorbic acid and surface area in expanding root tip cells suggest that it may be a constituent of the middle lamella, the primary cell wall and the cytoplasm   The indophenol-reducing activity of the ascorbic acid in the wall may be preserved during the phases of

expansion where the potassium and possibly magnesium to calcium ratio is presumably considerably higher and the phosphorus to nitrogen ratio slightly higher (112, 113) than later when maturation processes become dominant

Allen (1) expressed the view that the middle lamella is not merely an intercellular substance of cement but that it may function as a "plastic region in the growing cell wall which is in a measure adaptable to the changing form and size of the protoplast itself, and to the firm resistant layers whose form must correspond to that of the protoplast at the time of their deposition " He also said that "the evidence seems to indicate that the pectic layer continues to increase in thickness about as long as the cambial cell is increasing in size   It is possible that the attainment by the cell of its adult size marks the limit of growth of the middle lamella.   In this case we might say that pectic acid is deposited so long as the metabolic processes of the cell result in a plus which is expressed in cell growth, but that later, when a metabolic equilibrium has been established, or when the excess of food is stored instead of being used for growth, or when the protoplast degenerates, a predominance of other cell wall materials is deposited The evidence for such a view, however, is far from complete "

The relation of ascorbic acid to growth of the cell wall was studied in the root-cell investigations previously mentioned (113)   It was observed that ascorbic acid increased approximately so long as the cell continued to expand During maturation a decrease was observed, becoming more marked as full development was attained   Ascorbic acid has also been found (Reid, unpublished data) as a constituent of young cotton fibers   Older, still growing fibers also contain it but in a lower concentration on a green weight basis   The content per cell at successive stages of development was not determined   Pectic substances have also been found in cotton fibers (53) and relatively more in young than in old fibers (148, 29)

Comparisons of differences in composition of cementing material of the walls in fungi and higher plants are of special interest with respect to possible inter relations of ascorbic acid and calcium in cell wall development   Fungi are said to contain little if any ascorbic acid and calcium is generally considered as unessential or, possibly, as essential only in very slight traces   It is known, however, that growth of some fungi, such as yeast, is much more rapid if calcium is present in the nutrient medium   It is possible also that biological tests for determining the antiscorbutic value of fungi may be difficult to conduct without danger of loss of ascorbic acid before the supplement is consumed Hence, small amounts of the vitamin would not be easy to evaluate by this method   In the higher plants both ascorbic acid and calcium are present and doubtless both are essential   Types of higher plants lacking chlorophyll require calcium but much less than chlorophyllous types, and they also contain much less ascorbic acid than the latter plants (43)   Chlorophyllous tissues such as leaves contain much higher concentrations of both ascorbic acid and calcium than the non green parts, with the possible exception of certain regions of extremely high metabolic activity as in the growing regions of roots   The reason why calcium and ascorbic acid may be essential in appreciable amounts for non green plants but

not for fungi is still obscure   It should be possible to determine whether or not differences in composition of the walls are chiefly involved   Pectic substances have been reported in the cell walls of cellulose-forming fungi (36) but the cement materials surrounding and binding the cellulose layers undoubtedly differ somewhat in chemical composition from those in higher plants   Presumably the pectic substances are not present as calcium salts since, as previously stated, only slight traces of calcium, if any, are essential for fungi   Calcium is found, however, in the walls of non-chlorophyllous higher plants   The general trend of evidence as to the distribution of ascorbic acid and calcium in different groups of plants definitely suggests the possibility of a mutual relationship to calcium pectate or to the calcium salt of some related pectic substance occurring in the middle lamella   Further study is necessary before drawing definite conclusions as to a direct relation of this vitamin to cell-wall development

The materials surrounding the cells of plant and animal tissues have been herein shown to consist of solid units bound together by a cement substance Carbohydrates or carbohydrate-containing complexes are important constituents of these binding materials, probably influencing strongly their hydrating and gelling properties   There is evidence that the cement substance in both types of tissue is a calcium salt, in the plant a salt of pectic acid, possibly linked to protein in embryonic cells and in the animal probably a proteinate, though it may be one with a carbohydrate complex

IV LOSS OF ASCORBIC ACID IN METABOLIC PROCESSES   1 *In plants*   Losses amounting to as much as 15 to 20 per cent in the absolute amount of ascorbic acid in young cowpea plants have been found to occur at night, but apparently only at temperatures which are favorable to growth (110, 111, 114, 115)   Other investigators have observed the same phenomenon in other types of plants (97, 133, 71)   The writer has observed similar losses also in older growing cowpea plants but, because of the larger and somewhat more variable sizes of the plants, the losses are somewhat difficult to determine quantitatively   Gains in the absolute amount of ascorbic acid during periods of darkness do occur so long as the stored food reserves in the cotyledons last   Following this period losses at night are found (114)   The evidence suggests that only seedlings and possibly sprouting tubers and bulbs with special types of storage reserves have the capacity to synthesize ascorbic acid during periods of darkness   It appears that plants at other stages of growth either lack the ability to convert some of their carbohydrate reserves into ascorbic acid or synthesis occurs but is not measurable because the losses are considerably greater than the gains

More recent work (116) affords evidence which supports the latter concept and definitely indicates an ability of plant tissues to synthesize ascorbic acid during periods of darkness   Tests were made with excised tomato roots (sixtieth explants) grown in a modified Pfeffer solution containing one per cent sucrose, thiamin (vitamin B_1), and pyridoxin (vitamin B_6) but no added ascorbic acid A definite indophenol-reducing action of the root extracts was observed with approximately the same speed of reaction as occurs with ascorbic acid and the reaction was of such magnitude as to eliminate the possibility of transference

of the total quantity from the original explants   From these results it seems fairly definite that excised tomato roots in sterile cultures have the capacity to utilize sucrose in the synthesis of ascorbic acid   It is also probable that roots of intact plants have the ability to form ascorbic acid at night by utilizing some of the stored carbohydrates but that the newly synthesized portion is not measurable because the losses are greater than the gains.   The nightly losses may thus be appreciably greater than the decreases as observed in the assays Presumably even greater losses occur in the daytime than at night but they are difficult to determine because the rate of synthesis tends to be greater than that of loss.   However, in rapidly growing plants with low carbohydrate reserves the writer has obtained indications of possible losses in the afternoon on cloudy days   The results thus suggest that ascorbic acid may play a much more important rôle in the economy of the plant than has heretofore been realized The fate of the disappearing ascorbic acid is as yet undetermined but, as previously suggested, it may possibly be used for a constructive purpose such as in the synthesis of some special constituent of the pectic substances in the growing regions   Support for this assumption is to be found in the apparent loss in total ascorbic acid in root cells during maturation (113) and also by the fact that at favorable temperatures a very rapid growth of roots usually occurs in young cowpea plants from the eighth to the tenth days, at which time there is a lag in the general upward trend of the curve representing the absolute amount of ascorbic acid in the plant (114)   Kohman and Porter (72) have recently reported that in tomato plants with roots severed the losses in ascorbic acid at night are less than in intact plants

The structure of the ascorbic acid molecule is such that it could presumably be converted into the uronic acid of d-gulose, but a compound of this type has not been reported as occurring in plant tissues   On the other hand it is possible that the loss of ascorbic acid which occurs in darkness may be, at least in part, a result of oxidation and indirectly a consequence of a lowering of the sugar content   It has been shown in *in vitro* experiments by Munilla and Vogelsanger (100) that sugars protect ascorbic acid from oxidation   A lowering of the sugar content might thus affect not only the synthesis of ascorbic acid but also its maintenance.   Results of other tests have shown that in green plants exposed to light, their ascorbic acid may be protected from oxidation by the chlorophyll (108)   Possible losses of ascorbic acid by oxidation are difficult to determine, at least in some types of tissue, because of the difficulty of complete removal of the hydrogen sulfide employed in reducing the dehydro form of ascorbic acid and also because a number of substances combine with hydrogen sulfide to form compounds which react like ascorbic acid (135)   Moldtmann (97) makes the unqualified statement, however, that the losses of ascorbic acid at night are not a result of oxidation   The need of further study of this problem is evident

Mention should also be made of the fact that plants grown in solutions containing high concentrations of basic ions (Ca, Mg or K) even when the nutrient medium is maintained at a favorable pH, have definitely, though, except in the case of magnesium, not markedly, lower ascorbic acid values than plants grown

in solutions containing medium or low concentrations of these ions   (104, 10, Reid, unpublished data) It is not known whether these decreases signify lessened synthesis or increased utilization   If, during the growth process, the vitamin undergoes a modification with eventual combination with basic ions, the latter hypothesis is more probably the correct one

2 *In animals*   Considerably less ascorbic acid is excreted than is ingested by the human body but no satisfactory answer is available as to where or how the loss occurs   Smith (134) in a review and discussion of the question of human needs of ascorbic acid, summarizes as follows the daily requirement in terms of milligrams per kilogram of body weight "Infants from 3 to 8 mg , children from 6 4 to 7 5 mg , and adults from 0 7 to 1 6 mg " The data are held to suggest that ascorbic acid requirements are related both to body weight and to metabolic rate, the effect of the latter being the more pronounced   None of the investigators who have studied the relation between intake and excretion of ascorbic acid have suggested the possibility of utilization of the disappearing portion for a directly constructive purpose   It should be pointed out that the period of maximum requirement of calcium is coincident with the period of most rapid growth and most rapid disappearance of ascorbic acid

There is considerable evidence that the utilization of calcium by the tissues is influenced by ascorbic acid (139, 64, 125, 34, 4, 82, 83, 75, 76, 84, 131)   Daniels et al (31), however, reported finding no relation between ascorbic acid ingestion of between 2 7 and 12 5 mgm per kilogram and calcium retention in children, and Henry and Kon (60) found no effect of an addition of 2 mgm daily of ascorbic acid on the retention of calcium in rats kept on a diet low in calcium   Mallon and Lord (89) observed that addition daily of 5 cc of lemon juice to young growing rats supplied with a diet containing 0 166 per cent calcium caused no increase or decrease in calcium retention   It seems possible, however, that ascorbic acid might influence calcium retention with a diet high in calcium Hawley, Frazier, Button and Stevens (55) studied the effect of acid and alkali upon the amount of ascorbic acid excreted in the urine of human subjects They found a pronounced decrease in excretion when the urinary pH was in the alkaline range of 7 5 to 8 1 and the effect was apparently not due to reversible oxidation   Tislowitz (138) reported that the injection of large doses of ascorbic acid into dogs caused a ten per cent increase in the alkali reserve   The results of other investigations suggest a partial explanation of these observations Robertson, Ropes and Bauer (121) studied the degradation of mucins and polysaccharides by ascorbic acid and hydrogen peroxide   They found that the system acted on starch, pectin, flaxseed mucilage, the polysaccharides of synovial fluids and cartilage and the capsules of various types of pneumococci but caused no apparent change in ovo-mucin, agar-agar or gelatin   Later work (122) by these investigators showed that the viscosity of gelatin also was reduced by this treatment   The question arises as to whether the breakdown of the macro-molecules may have been influenced to some extent by the removal of traces of calcium and possibly of other bases from the reacting materials   Grant (45) presented evidence which shows that mucus, a glycoprotein, is an immediate

source of calcium in gastric secretions and that it constitutes an important factor
in the reduction of gastric acidity The presence of varying amounts of mucus
in secretions of different types is held by this investigator to be responsible for
calcium and acidity variations in gastric secretions The work of Oden (102)
suggests that the carbohydrate moiety of the mucin molecule may well be in
volved in these pH regulatory reactions He obtained evidence that pectic
substances may act as regulators of the H and OH ion content of circulating
solutions in plant tissues in so far as these come into contact with the pectic
materials That pectic substances may exert some as yet unidentified beneficial
influence in the digestive tract in addition to their action as lubricants is generally
recognized (70 144, 145) Werch et al (145) suggest that an effect of pectin
on the pH of the intestinal contents is the most probable explanation of the
beneficial action of pectin in the treatment of diarrheas caused by some types
of intestinal pathogens

Losses of ascorbic acid are herein shown to occur in both plants and animals
Metabolic losses occur during growth in some, if not in all plants, and in ani
mals, the losses are much greater during rapid growth than in the adult stage. It
is suggested that ascorbic acid may be used in the synthesis of some substance
essential in growing regions There is considerable evidence that the utilization
of calcium in the tissues is influenced by ascorbic acid and conversely, the utiliza
tion of ascorbic acid by the tissues presumably is influenced by calcium

V SIMILARITY IN SOME PHYSIOLOGICAL EFFECTS OF ASCORBIC ACID AND
CALCIUM 1 *In animals* a *Growth* The necessity of ascorbic acid for
growth of higher animals is generally recognized but direct experimental evidence
of such a relationship is somewhat limited Young animals succumb to its
absence in the diet so suddenly that a retarding effect on growth is often not
observable Anderson and Smith (2) in paired feeding experiments with guinea
pigs found that animals given scurvy preventing supplements and whose food
consumption was iso-caloric with that of scorbutic animals attained a greater
weight A relationship of ascorbic acid to growth of cells is suggested by results
reported by Glick and Biskind (44) They observed that the size and ascorbic
acid content of the cells of the adrenal cortex increased regularly from the fetal
to the adult stage of the animal The necessity of calcium for growth has long
been recognized Boelter and Greenberg (19) have recently presented further
proof that growth is retarded when the supply of calcium is low

b *Water relations* Calcium and ascorbic acid are probably jointly involved
in exerting an effect upon the water content of cells and tissues The extremely
rapid losses in weight often observed in guinea pigs with severe scurvy could
presumably be a consequence of an upset water balance associated with a
disturbed equilibrium between the solid and liquid cytoplasmic phases resulting
in lowered ability of the cells to retain water It would be of interest to find
whether or not at a stage preceding the final collapse there was not increased
hydration of the cell colloids as a consequence of release of calcium from the
intercellular cement McHenry, Reedman and Sheppard (95) and Sheppard
and McHenry (132) found in feeding tests with guinea pigs that lack of ascorbic

acid caused a diminished retention of water which largely accounted for the observed differences in body weight   They concluded that ascorbic acid is apparently concerned directly or indirectly with water retention   Ley (78) described a condition in very young infants, characterized by a disturbance in water metabolism and marked loss in weight which responded quickly to administration of ascorbic acid

It is possible that the therapeutic value of ascorbic acid in fever-producing infections may be a consequence of a beneficial influence upon the water balance [1] Balcar, Sansum and Woodyatt (9) suggested that "fevers are due to a deficit of free water resulting in an abnormal tendency on the part of the colloids of the body to bind water "   They held that the poison of the disease leads to changes in the cell colloids, increasing their hydration capacities, so that they take up and bind more water   They suggested that beneficial results would follow if enough water were introduced to saturate the increased affinities of the colloids and provide excess free water   There is considerable evidence that ascorbic acid may reduce the water-binding capacity of certain types of proteins   Pozzi (107) has shown that it decreases the viscosity of gelatin solutions and McClean and Hale (94) and Madinaveitia and Quibell (86) have reported similar effects on mucin preparations from vitreous humor   The work of Robertson, Ropes and Bauer (121, 122) previously mentioned has shown that the reduction in viscosity on treatment of gelatin, synovial fluid, gastric and salivary mucins with ascorbic acid and hydrogen sulfide causes a reduction in viscosity which is irreversible, due to a breakdown of macromolecules   Honaga (65) reported that in most diseases causing fever the blood calcium is lessened at the height of the fever whereas with blood potassium the opposite occurs   During convalescence normal values are restored   He found also that induced fever in the rabbit increases the calcium content of the liver and muscles and decreases that of the skin   The increase in calcium in some tissues during fever may possibly be principally intracellular and the loss from others may be chiefly from extracellular sources   The increase in calcium content of certain tissues suggests that the calcium may be directly involved in a hydrating and gelling action upon the cell colloids   The favorable action of ascorbic acid may be a consequence of release of the bound water and removal of the excess intracellular calcium

c *Permeability*   Chambers (24) described three types of cellular membranes in multicellular organisms, in the first of which the intercellular cement is present in such an appreciable amount that the permeability of the membrane is determined by it rather than by the cells   The endothelial lining of the blood vessels is an example of this type   Permeability of the second type of membrane is determined by both the intercellular cement and the cells, the intestinal mucosa being an example of this type   In the third kind of membrane the cells fit together so closely that the cement has a negligible influence on permeability   An example of this type is to be found in the walls of the proximal tubules of the kidneys   It has been previously shown in this discussion that the intercellular

[1] This suggestion was made to the writer by the late Dr Lillias D Frances

cement, at least that of certain epithelial tissues, is a calcium salt, the formation and, presumably, also the maintenance of which is dependent upon the presence of ascorbic acid. It seems probable that membranes of the first type should be affected most severely by shortage of essential cement materials. If either calcium or ascorbic acid is lacking, extravasation of both blood cells and fluids occurs especially if the tissue is subjected to extra strain or pressure. When both calcium and ascorbic acid are deficient, earlier and more pronounced histological defects would presumably be expected. Excessive intake of either ascorbic acid or calcium with an inadequate supply of the other would possibly be harmful. Because of the apparently interrelated action of calcium and ascorbic acid it might be expected also that a deficiency of either of these substances would cause some of the symptoms characteristic of lack of the other. This, in fact, does occur. The results obtained by Boelter and Greenberg (19) have shown that shortage of calcium causes retardation of growth, reduced activity, marked weakening of the muscles and paralysis of the hind legs, high basal metabolic rate, internal hemorrhages, and osteoporosis, all of which are characteristic scurvy symptoms.

d. *Detoxifying action*   There is considerable evidence that ascorbic acid protects the body against the toxic effects of certain chemically unrelated substances such as lead, benzene, arsenicals, some types of amines, and barbiturates but the basis for a chemical action has not been found. It is possible that physical-chemical factors which may influence surface action may be chiefly concerned. If this is true, and if the influence of ascorbic acid is exerted principally at or near cell surfaces, as herein suggested, it becomes unnecessary to postulate specific chemical reactions with these substances to explain the detoxifying effect.[2]   The suggestion has also been made that the detoxifying action of ascorbic acid against certain chemicals may be related to a tightening effect upon either or both the plasma membrane and the cement substances in the cell walls. Such a change in either of these structures would tend to retard the passage of large molecules.[3]   Undoubtedly calcium would be involved in this and in some of the other detoxifying reactions but present knowledge is insufficient to warrant making a positive statement regarding such an influence. The beneficial action of ascorbic acid in alleviating the toxic effects of lead can probably be explained as a precipitating action on the lead. It should be added here that if the detoxification of some types of substances is a surface reaction, synthesis of ascorbic acid may be a surface phenomenon also, since the two processes appear to be related according to evidence presented by Longenecker, Fricke and King (81).

e. *Muscle action*   Friedman (39) studied the effect of ascorbic acid deficiency on smooth muscle responsiveness to stimulation with histamine and found a greatly reduced reaction as compared to that in normal tissues. Basu and Biswas (13) and Basu and Ray (14) studied the influence of ascorbic acid on different types of muscles and found that it augments contraction, brings about

[2] This suggestion was made by Mr. Chas. H. Binkley
[3] This suggestion was made by Dr. L. E. Yocum

quicker rela\ation and delays the onset of fatigue   Their experiments showed also that the effects are not due to any alterations of pH   Results also of tests reported by Gantenbein (41), Matthes (92), and Linstead and Krayer (80) indicate a stimulating effect of calcium on muscle action   Lack of calcium also affects the muscles causing loss of tone and flaccid paralysis   Basu and Biswas pointed out that the response to ascorbic acid is somewhat similar to that to calcium (Gellhorn, 42)   The present concept of muscle action is said to be that "calcium is released on excitation from its combination with protein and this released calcium causes reversible gelation in the interior of the protoplasm "   If calcium is added to the external fluid, the gelated condition is induced much more readily and to a greater extent than before, and consequently the response is greater   The suggestion was made that since ascorbic acid is known to cause gelation in different fluids, it is probable that it also causes gelation in muscle plasm   The suggestion could also be made that ascorbic acid aids in the recovery process and influences intracellularly the solation phase of the reaction

The possibility of interrelated effects of ascorbic acid and calcium on other tissues in which surface activities play an outstandingly important rôle such as those of the nervous system, glandular organs and white blood corpuscles has not been investigated with this particular aspect in view

f   *Effect of ascorbic acid and calcium on catheptic enzymes*   Additional reason for postulating an interacting relationship in the functioning of calcium and ascorbic acid is to be found in the fact that both of these substances have an activating effect on catheptic proteolytic enzymes (68, 33, 5)

2   *In plants*   Direct proof of the growth relations of ascorbic acid in higher plants is difficult   The same is true also of a possible connection of this vitamin with other physiological functions because it is always present in metabolically active tissues   If, as some of the reported observations suggest, ascorbic acid functions in the cytoplasm and at surfaces of cells, as has been previously shown for calcium, it may presumably exert an important influence upon the intake and retention of water and also of mineral nutrients   It has been shown (137, 63) that absorption of mineral salts by roots occurs chiefly during active growth or in roots with the potentiality for active growth   It has also been demonstrated (106) that the maximum penetration of potassium nitrate into soybean roots occurs between 1 and 4 mm from the tip   The penetration of salts was found to diminish progressively toward the older region of the root and to stop almost completely at 10 mm   Osterhaut (103) observed that ammonia entered the fresh surface of Valonia cytoplasm squeezed out of cells and stated that it did so by forming a compound with an acid at the surface   The available evidence indicates that the region of active growth and absorption in roots is identical with that having the highest ascorbic acid concentration per unit area of cell surface   The general trend of evidence strongly suggests the possibility of the movement of water and mineral nutrients through the cementing material of the walls in the growing region of the root in much the same fashion as materials are transferred through the cement substance surrounding the endothelial cells of the blood vessels in animals

SUMMARY

Calcium gives stability to cell surfaces and intercellular substances of both plant and animal tissues and there are indications that it may act in somewhat similar manner in the two groups

Ascorbic acid is necessary for the production of certain types of intercellular materials, and possibly also for the production and maintenance of cytoplasm and normal plasma membranes in animals and there is considerable suggestive evidence that it may perform a similar function in plants   Whether its primary action is to influence the functioning of cells or the production of intercellular colloids or whether it is involved in both of these types of activity has not been fully determined

Similarities are shown in the physical constitution of the cell walls of plants and the intercellular substances of animal connective tissues, namely, that they both contain solid units, different as to chemical nature, and bound together by cement substances containing units which are chemically somewhat similar It is supposed that in plants the latter materials may aid in the maintenance of a plastic state during expansion and later, on setting firmly, impart strength to the structure   The plastic state presumably is maintained by retarding pre cipitation of calcium whereas the maturation phase of the process presumably allows freedom in calcium deposition   There is suggestive evidence that a somewhat similar sequence of changes in form of the intercellular substances occurs also in some types of animal tissues

Collagen, which is concerned in the gelling action of the intercellular matrix in animals, contains sugars   The mucoid like ground substance surrounding the collagen fibrils is held by some investigators to contain both sugars and uronic acids   Sugars and uronic acids are present in plant tissues as constituents of the pectic materials, complex carbohydrates with marked hydration and gelling properties   Supposedly calcium plays a rôle in the gelling action of these sugar uronic acid complexes

Ascorbic acid exhibits properties which may adapt it for functioning at cell surfaces, namely, it shows definite surface activity in lecithin and cholesterol solutions, and it combines readily with protein in the presence of calcium Presumably it could act as a carrier of calcium in embryonic regions, it could also be involved in preventing premature calcification, and its structure is such that it could probably itself be changed to form a special component of the pectic acid molecule

The increase in surface of expanding cowpea root tips at successive stages of development approximately parallels the increase in ascorbic acid   During maturation the values become lower which is interpreted as possibly indicating a transformation of the vitamin to a non reducing substance during maturation processes   It is suggested that ascorbic acid may be used in the formation of calcium pectate or a related substance.   Further suggestion either of the utiliza tion or the destruction of ascorbic acid in plants is supplied by the fact that under conditions suitable for growth, marked losses of the vitamin occur in plants kept in darkness and presumably also in those kept in light   There are

unaccountable losses of ascorbic acid from animals also, though the magnitude and manner of loss are difficult to determine

In addition to the parallelism between ascorbic acid and cell surface increases in growing root cells, further suggestive evidence that vitamin C may be concentrated at cell surfaces is as follows   Cell surfaces of plant tissues contain substances with an acid reaction, a rapid reduction of indophenol occurs when roots of plants with tops exposed to light are immersed in the dye solution, and in animal tissues there is considerable tendency for deposition of silver granules to occur at cell boundaries and in intercellular spaces as well as in the cytoplasm when the tissues are placed in acidified silver nitrate solution

The very low requirements or possibly unessential nature of ascorbic acid and calcium in the development of fungi and the essentiality of calcium and probable essentiality of ascorbic acid in higher green plants is pointed out as is also the fact that calcium pectate is a constituent of the walls of higher plants, even of non-chlorophyllous types, but is not necessarily present in fungi

The availability of relatively high concentrations of calcium, magnesium and potassium appears to have a favorable influence upon retention of ascorbic acid in animals as evidenced by lessened excretion   High concentrations of these elements cause lower values in plants, but it has not been determined whether the difference in content is due primarily to decreased synthesis or to increased utilization

Glycoproteins such as mucus may serve as sources of calcium in gastric secretions and there is suggestive evidence that the carbohydrate moiety of the mucin molecule may be involved in pH regulatory reactions

Similarities are shown in the physiological effects of calcium and ascorbic acid with respect to water relations, permeability and growth in plants and animals and also to their effect on muscular action and on catheptic enzymes in animals   Ascorbic acid exerts a detoxifying action in animals against a number of substances   There is as yet little more than suggestive evidence that calcium may also be involved in these reactions

If ascorbic acid and calcium function co-operatively as has been shown in maintenance of the intercellular cement in the endothelium of the blood vessels, it should be expected that there would be marked similarity, although not necessarily complete identicality in symptoms when one or the other of these substances is lacking   The observed similarity in some of the deficiency symptoms of the two substances affords support for this assumption

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MALIGNANCY IN RELATION TO ORGANIZATION AND DIFFERENTIATION

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Malignancy was tentatively formulated in a succinct report by the national cancer committee (Bayne-Jones, 1937) as a universal cell potentiality, varying in degree with cell type, of the nature of a somatic mutation, and resulting in automatic proliferation independent of a continuously acting provocative agent It was asked "have they (the malignant cells) become 'fast' to the conditions which normally control cell growth in the body or is there a break in the internal control mechanism of the cell, or is there a loss in body control of cell activity? This, the core of the problem, has been almost entirely neglected "

The apparent neglect is clearly not lack of consideration It is due to the standards of reference also being problems as challenging as malignancy itself Malignancy and its associated phenomena merely constitute one aspect of growth, proliferation and differentiation of cells and cell constituents The nature of the growth controlling forces of the body, of the internal control mechanism of the cell, the relation between nuclear change and cytoplasmic and organismic expression, the determination and maintenance of cellular specializations, and the mode of reproduction of proteins, mitochondria, enzymes, genes, and viruses are as little understood and equally challenging The problem of malignancy becomes one of synthesis involving the problems of development as a whole Clarification at any point is likely to throw light on the whole interrelated structure.

The outstanding feature of malignancy is that tumor growth is uncontrolled in relation to the host and shows little or no organization within itself It is remarkably like that of normal tissues cultivated outside the body which grow in the same inchoate manner, tending to form marginal metastases and exhibiting a similar apparent decrease in degree of differentiation In the sense used by Child (1915) malignancy becomes physiological isolation

1 Growth controlling and organizing forces The nature of the agency controlling and organizing the growth and multiplication of cells in an organism is obscure although its existence seems to be established (Sinnott, 1939) In the organism each cell develops in conformity with the character of its surroundings Cues are decidedly of supra-cellular origin (Weiss, 1940) The unlimited proliferation when cultured *in vitro*, the definite limits to growth of an organism under optimal nutritional conditions, the limits of growth and nature of regenerating tissues, and above all the growth rate curves characteristic of all organisms and of their constituent parts all indicate a subordination of cells within the organism to a control by the whole

Various suggestions have been made concerning the nature of the controlling

agency but they suffer generally from vagueness and intangibility verging on the metaphysical   These, the field theories of embryology (Weiss, 1935, Waddington, 1935) have an undoubted descriptive value and are symbolic of a real quality of the organism, but the only attempts to translate the philosophic conception into chemical or physical terms, the metabolic gradient (Child, 1941) and the electro-dynamic theory (Burr and Northrop, 1935), have not met with ready acceptance   Yet it is startling to see the pattern of an organism change under the impact of a single substance, such as thyroxin upon amphibian metamorphosis   The field idea remains potent

Field action may be considered as a tendency toward a certain equilibrium (Huxley, 1935)   Even the mathematical rules governing the rate of regeneration bear a resemblance to those governing the restoration of electrical equilibrium between two regions at different potentials (Przibram, 1917)   A limbbud grafted in abnormal position after the dorsoventrality is determined will undergo a gradual rotation toward a normal orientation, now that it is no longer sufficiently plastic to have the polarity of the whole imposed directly upon it (Harrison, 1921)   Whether or not the controlling agency is a product of the cells it controls, cells or cell groups in an organized system acquire something distinctive of their position in the system

It is commonly the case in regeneration from fragments for the regenerating tissue to proceed at a steadily decreasing rate until the original piece comes to occupy the same relative position in the new organism as it did in the old   In a significant number of cases there is a precision to a degree far beyond any biological need and unrelated to visible structural expression (Allen, 1921) Fragments of Hydra, placed more or less in contact, slide into positions so that each piece reoccupies approximately its original level in the organism (Papenfuss, 1934), while in everted Hydras the cells of the inner and outer layers slide between one another to regain their proper respective positions (Roudabush, 1933)   Dissociated sponge tissues exhibit similar phenomena (Wilson, 1911, Brien, 1935, 1937)   These examples are admittedly from lowly organisms but it is only in organisms of an order of size within range of cell dimensions that such phenomena can be expressed

To what extent is this quality related to cell specialization?   It is evident from the behavior of Hydra fragments and sponge dissociates that like cells and like tissues mutually adhere, and repulse unlike cells and tissues, while even homologous cells and tissues fail to cohere when taken from different species of sponge (Wilson, 1911) or Hydra   Specific and even racial differences in amphibians and in mammals may inhibit successful transplantation of tissues in mature animals, although embryonic tissues from different classes of vertebrates may be grafted together at least for a time   In amphibians a tissue incompatibility develops finally that significantly inhibits even autologous grafts (Harris, 1941)

It is significant that mammary tumors of mice exhibit similar compatibility phenomena, grafts within a given strain attaining almost 100 per cent success but very few to mice of another strain (Lewis and Lichtenstein, 1936)   Barnes and Furth (1939) found that a mouse tumor originating in the reticulo-endo-

thelial system could be transplanted to every mouse of the ancestral leukemic stock but to no mouse of the ancestral non leukemic stock, although it would grow steadily on chick allantois A somewhat similar situation exists for cellular constituents While gonadotropic hormones of amphibia seem to be without specific effect, there undoubtedly exists a species specificity The effectiveness of the hormones in a foreign species tends to vary directly with the phylogenetic proximity of donor and recipient, and the antibody nature of the sera is probably produced as an immune reaction to a foreign protein (Creaser and Gorham, 1939)

The protein basis and uniqueness underlying such phenomena probably relate in some way to the nature of the controlling agency Yet during development the controlling system precedes cell and tissue specialization

If malignancy is to be considered as a loss or weakening of the control of cell activity by such a system it is improbable that one cell should alone escape from the control Multiple foci should be the rule rather than the exception If malignancy is primarily due to a weakened control rather than an intrinsic change in the cells, transplantation of tumor cells to normal healthy hosts of the same species and race should fail to perpetuate the malignant growth Such grafts should be incorporated or absorbed by the host system

In frog embryos derived from overripe eggs tumor like growths develop early, overrun and kill the embryo within a few days (Witschi, 1931) This and related phenomena led to an attractive speculation by Needham (1936) that overripeness of the egg leads to an upset of sterol metabolism, in turn leading to deviation of sex hormones upsetting the sex ratio uncontrolled liberation of the embryonic evocator resulting in monster formation and the production of chemically related carcinogens leading to epitheliomata

More recent work confirms the observation that while multiple tumors do develop in the primary host embryos they are due to a true weakening of the embryonic individuation field and a general reduction of proliferation rate The tumors are the products of those cells that continue to divide at the normal rate, multiplying more rapidly than adjacent tissue and so assuming the form and effect of tumors (Briggs, 1940, Briggs and Berrill, 1941) When such a tumor is grafted into equivalent embryos of the same species it grows at the same pace as the surrounding tissue, loses its papillomatous character and is slowly absorbed by the host like many other grafts of normal tissue

Weakness of extrinsic factors in the embryo therefore leads to a real malignant behavior by some parts, but there is no intrinsic malignant quality in the tumor tissue that can be expressed in a normal system

2 Histogenesis and tumor extracts There is considerable evidence that histo differentiations generally develop according to location in the system Position effects must operate so that a cell is directed along or made to select among its repertoire of potential specializations The variety of special types varies with each kind of organism, but each kind remains distinct without grading into other types

While the study of regenerates emphasizes the impact of position upon cell

destiny, an alternative mechanism is present in developing eggs, especially those of determinate or mosiac type

It is generally considered that the inductors of vertebrate embryos are diffusible chemical substances   In the case of determinate eggs such substances arise precociously before significant diffusion becomes necessary, and are known as "organ-forming substances" (Conklin, 1905, 1929)   Primarily they are specialized regions of the egg cytoplasm that are able to induce such rapid histogenesis that cleavage in these territories becomes inhibited and is limited to a few divisions (Conklin, 1905, Berrill, 1935)   The inductors are liberated or activated at the time of rupture of the matured egg nucleus and resulting mixture of nuclear sap with the cytoplasm

The inductor in amphibian embryos is considered by some to be a sterol (Waddington, Needham, 1936), by others to be protein (Barth and Graff, 1938), but more recently Holtfreter (1942) finds that all known agents act indirectly by causing cells to release the active substance the nature of which is completely obscure

Sex hormones appear to act histogenetically and are known to be steroids They are produced by gonadial tissue, even that of echinoderms (Donahue and Jennings, 1937) producing estrin as in the case of mammals and other vertebrates   Tissues in widely different parts of the body respond to these circulating secretions   According to Burns (1938) the sex inductors of amphibian larvae are identical with the sex hormones of the adult, and in these young forms they act through diffusion from cell to cell as do the inductors in the embryo   Each component of the developing ovotestis secretes its own hormone, to which it has a specific growth response   The hormone diffuses into the other component and the one that finally overrides determines the sex   Such substances are not specifically histogenetic since they arise in and affect tissues already more or less specialized   But it is significant that relatively specialized tissues secrete specific substances that promote the growth of the parent tissue   A similar case is the production of biotin by yeast, stimulating but not necessary to the growth of yeast

The tissue response in these two instances is much like that of the circulating hormones   The immediate response in every case is an increase of proliferation in the tissue sensitive to the hormone   In most cases there is a specific differentiation   The specificities however appear to be associated with the responding tissue rather than the inducting substance   The inducting steroid is in the general circulation, reaching all tissues, but only certain related tissues are sensitive to it, while the histodifferentiation response is specific to each tissue responding   It is difficult to determine whether the specific differentiations are induced directly by the steroid or by the steroid-induced proliferation

Hormones generally may be considered as tissue extracts, some originating in what appear to be residual tissues whose primary functions have been lost (Huxley, 1929), for example, thyroid from endostyle   Experimental support for this concept comes from Helff (1926) who found that perforation of the frog operculum enabling leg emergence is due to a hormone produced by underlying degenerating gills, and less intensely by tissues in general undergoing histolysis

Zuckerman (1940) concludes that the idea of specific action of estrogenic hormones must be abandoned   He suggests that true Mullerian tissues respond by glandular proliferation and the epithelial metaplasia and stratification is anatomically a response of tissue in whose development estrogen-sensitive sinus epithelium has played a part

The response is however even more general   Estrogen accelerates and intensifies the breakdown and ossification of growing cartilage, whereas the thyroid hormone increases proliferation and differentiation, with retarded ossification, of the same tissue (Silberberg, 1939)   Histospecific responses appear therefore as the result of intensifications or retardations of each respective tissue cycle, as in the response of feather follicles to estrin and thyroxin (Lillie and Juhn, 1932)

Tissue cultures in vitro exhibit a loss of viable differentiation, related to the enhanced proliferation rate   This might be called dedifferentiation except that such tissues redifferentiate only their respective original character upon reimplantation (Strangeways, 1924)   Mitotic multiplication inhibits the full expression of cell character, just as enforced differentiation inhibits cleavage (Berrill, 1935, Bloom, 1937)

Something persists through successive divisions in spite of the loss of structural and secretory indices, that maintains specific character   Either irreversible changes occur in the nuclei of specialized cells even though nuclear equivalence has been demonstrated in early developmental stages, or some substance is produced in the cell during the original differentiation that is not only an associate of its particular type of specialization but can act as an inductor of that special type   It may act directly on the cytoplasm or indirectly through nuclear activity

Tissue maintenance factors and the so-called organ forming substance of eggs may be identical and in effect be specific cytoplasmic selectors of nuclear repertoire, formed in the first place by nuclear reaction with relatively indifferent cytoplasm under the influence of a supracellular selector

Malignant tissues frequently have been described as dedifferentiated or having reverted to an embryonic condition   In view of the real state in tissue cultures however it is clear that the high proliferation rate of tumor cells has a similar effect upon visible differentiation

The question whether malignant cells have lost or gained something is relevant here, although it raises almost every aspect of cell function and constitution   Many instances are recorded of normal secretory functions persisting in malignant tissue   Mouse hepatoma secreted bile in primary and transplanted tumor (Strong and Smith, 1936)   Fibroadenomata of rat breast secreted milk (Grauer and Robinson, 1932)   Cartilage tumors produce a more or less normal ground substance (Purdy, 1938)

It is evident that malignancy in itself does not necessarily mask or destroy the basic cell character   If malignancy is the expression of something lost from the cell, the loss appears to be unrelated to the determinant of cell type

The most striking examples of histogenetic tissue extracts come from malignant tissue itself   The reason for this is obvious   Malignancy implies unlimited growth   If to this is added a histogenetic effect, this effect will be ex

hibited on a relatively enormous scale, although the malignancy has generally tended to overshadow this other quality   The transmission is exact, the same cell types, macroscopic features, etiological habits and degree of malignancy are induced by cell free extracts in the host as they appeared in the original tumor The most obvious cases are those of tumors with the more striking histological character such as cartilage or bone   Cell free extracts of chondrosarcomas and osteochondrosarcomas of the fowl transmit their specific histology as well as malignancy (Rous, Murphy and Tytler, 1912, Muto, 1915, Furth and Breedis, 1937)   Similar induction of malignancy together with specific histological character by cell-free tumor extracts has been demonstrated in mice (Parsons, 1936) and with less certainty in the frog (Lucke, 1938, Briggs, 1942), the former case from chemically induced primary tumors

Malignancy in this light becomes one of the many properties of a cell induced in host cells by its noncellular extract   Similar extracts of nonmalignant cells might have similar induction properties, but without simultaneous conveyance of malignancy and consequent growth they remain indetectable

Both components of a reacting system must be considered   Specific induction is to be correlated with a specific response   There appears to be a definite relative sensitivity of host tissues to extracts of related tissue types (cf Barnes and Furth, 1939)   Specialized cells are apparently unable to undergo true dedifferentiation or to acquire the power to redifferentiate along new line   The least specialized cells are likely to proliferate most since specialization and mitotic activity seem to be competitive exercises   Otherwise the induction-response system appears to be fairly definite   In chick embryos or membranes induction by sarcoma extract occurs only when in contact with mesodermal tissue   In adult fowl connective tissue cells react to sarcoma extract to reproduce the particular type of sarcoma extracted   The response is chiefly from sites of tissue derangement such as actively functioning ovary when injected intravenously or along the path of injury when into breast muscle   Muscle from susceptible fowl absorbs Rous tumor I agent, muscle tissue from nonsusceptible and nonmesenchymatous tissues from susceptible fowl do not (Claude and Murphy, 1933)   Fowl endothelioma extract acts upon endothelial cells (Begg, 1927) According to Schiller (1937), a carcinomatous center infects adjacent epithelium but not connective tissue   The mammalian reticuloendothelial system reacts to extracts of lymphosarcomas (Parsons, 1938), while in frogs extracts of kidney adenocarcinoma appear to induce similar carcinomata in growing kidney tubules of other individuals (Lucke, 1938, Briggs, 1942)   Lyophylized filtrates of mouse mammary tumor taken by mouth induce mammary tumors in AX strains (Bittner, 1941)

In general there appears to be a relative sensitivity to tumor extracts of those less specialized tissues most closely related to the tumor tissue type   To this extent there is a close parallel with the endocrine-tissue reacting systems, and the problem may be one and the same

Induction by tumor extract has been considered to be species specific, both for birds and frogs   If the parallel between the tumor extract reaction and sex

hormones reaction is significant species specificity is to be associated with the carrier or protein component rather than the lipoid element Tissue specificities develop during the course of development (Harris, 1941) Tissues can be blended in early stages that mutually reject one another at a later period

Rous sarcoma extract normally fails to induce any significant response in ducks, but when massive injections are made into newly hatched ducklings malignant disease is invoked Resistance becomes complete within a day or two of hatching The question of susceptibility and resistance to tumor extract is probably as closely associated with protein specificities as is the degree of resistance to the grafting of normal and malignant tissues Duran Reynals (1942) reports more than an adaption of fowl sarcoma extract to ducks. In the chick the agent induces sarcomas and lymphosarcomas especially of liver and spleen but in the duck the same agent when successfully adapted produces wide spread tumors of skin and digestive tract, and also growths in the skull, ribs and muscle, that is, primarily affecting epithelial and to a lesser degree mesenchymatous tissues. Adaptation to duck results in chick incompatibility, and the same procedure is necessary to produce reactions in the chick as is necessary to overcome duck resistance in the first place The duck modified agent now produces in the chick multiple bone tumors from periosteum and endosteum that invade the marrow cavity, also inducing nonmalignant new bone in the non invaded parts

The question arises whether the agent is actually a histogenetic inductor whose specific type is alterable or whether it merely stimulates various tissues, according to their constitution to proliferate and produce their respective growth types

3 Teratomata Teratoma is among the most complex and challenging phenomena of malignancy It may be defined as tumors exhibiting little or no organization but containing many of the specialized tissue types The phenomenon goes to the base of the problem of histogenesis and is clearly one of a malignant tumor exhibiting in part epigenetic development The problem is threefold how do the specialized tissues arise, what is the significance of the apparent absence of organization, and what is the significance of the malignant character of the growth An adequate answer to all of these would raise embryology to the status of a classical science

Barron (1916) described pineal teratoma as consisting of muscle fibres, goblet cells, cartilage, bone, glandular, squamous and columnar epithelia Similar tumors are reported arising in thyroid (Putsch and Nelson, 1935) and suprarenal tissue (Geschichter, 1935), and more generally from gonadial tissue of either sex The limited mixed types usually containing cartilage described for the palm (Simard 1937) breast (Binkley and Stewart, 1940) and liver (Bullock and Curtis 1925) are probably aspects of the same phenomenon

Teratoma testis has been produced experimentally by a number of workers (Bagg 1936, Fahn 1940) through the combined action on the testis of zinc salts and normal or injected sex hormones According to Champy and Lavedin(1939) seminomas and embryomas may be induced by almost complete removal of the

testis A typical tumor consists of a stroma of embryonic mesenchyme which forms hyaline cartilage, often replaced by bone of endochondral origin, and muscle, glandular ducts lined with goblet cells or columnar epithelium, islands of keratinized stratified epithelium, feather follicles with feather germs, and medullated and nonmedullated neurones, with obvious areas of carcinoma and adenocarcinoma Needham (1936) compares it to the chaotic condition when an individuation field is thrown out of existence, when a "field" at some point fails to control evocator (inductor) substances Falin (1940) considers that at the site of zinc injection necrohormones, trephones, etc , are liberated during cellular disintegration that act on pluripotential testicular cells like the inductors or evocators of embryos, and that lagging or misplaced gonocytes are responsible for all teratomas He criticizes Michalowsky's (1932) suggestion that teratoma testis arises from spermatogonia and that the spermatogenic cells of the tubules approach the ovum in capacity He finds it necessary to assume parthenogenesis of spermatogonia and finds it difficult to account for teratomas elsewhere

According to Holtfreter's recent work (unpublished) the embryonic inductor responsible for neurulation is liberated from damaged cells, as imagined by Falin, but this inductor is not considered by embryologists to initiate development as such, nor is parthenogenesis conceived as applying to any cells other than fully grown ova in the throes of polar body formation Experimental or natural parthenogenesis has never been observed in oogonia or partly grown oöcytes

Two kinds of cells have the capacity for complete or partial development, fully mature ova and entirely unspecialized totipotent cells These types are poles apart and their confusion is responsible for much loose terminology used in the interpretation of teratoma

Spermatozoa and mature ova are each in their own way extremely highly specialized types of cell It is part of the special nature of the fully formed egg that when properly stimulated it undergoes rapid segmentation as a consequence of its large size, and simultaneously undergoes a series of specialized form changes associated with regionally developed special cytoplasmic differentiations (cp Needham, 1933, Berrill, 1935) Together these produce the embryo with its peculiar characteristic early stages Immature ova lack both properties and have no capacity as such for development To speak of parthenogenetic development of either oögonia or of spermatogonia is in terms of embryology nonsense

There is presumably a phase, however, in the production of oögonia and spermatogonia when the cells are entirely unspecialized, before even their respective destinies to produce oöcytes and spermatocytes have been determined Such unspecialized types of cells may exist in many parts of the body In lower vertebrates and most invertebrates unspecialized tissue consisting of multipotent or even totipotent cells occur throughout the organism, and are responsible for the regenerative capacities of these forms Except for the progressive specialization of tissues in higher vertebrates, there is no *a priori* reason why such unspecialized types should not persist locally and in small numbers in these as well

Under certain circumstances such cells or cell groups are capable not only of regenerative development, as in amphibians, but of total development as in tunicates   The course and conditions of development however are not the same as those of developing eggs   Physical or physiological isolation from the parental organism is essential for total development in the one case, similar isolation of the part from the whole is necessary in the other   Then if proliferating cells are able to adhere together and are continuously supplied with adequate nourishment, development of organized differentiating structure proceeds   It is necessary to emphasize that in the absence of a relatively large quantity of material to start with, as is present in even the smallest egg these cells divide because they have grown beyond the normal limit and are permitted to do so by their immediate cellular environment   The invocation of inductors or evocators is neither more nor less serviceable than it is for all malignancy   They may be necessary to account for certain kinds of histogenesis even in teratoma   but only of histogenesis of cells already present or multiplying   They do not account for growth itself

In virtually every account of teratomas the fact of malignancy is more or less taken for granted, the assumption being that parthenogenesis of mature ova in the ovary, or the miscalled parthenogenesis of öogonia and spermatogonia, account for the type of growth   Distorted development may be explained by the abnormality of the site of development, but teratology is not malignancy   Either malignancy develops in one or more tissues as development proceeds, which is conceivable, or malignancy exists from the beginning and development is its outcome

There is little doubt that on occasion mature ova develop parthenogenetically in the ovary, for the most part forming benign teratological growths   Under abnormal developmental conditions spontaneous malignant changes might well take place in one or more tissues   The problem would be that of spontaneous tumors generally

In the case of teratomas appearing at other sites parthenogenetic development, even if it had meaning here beyond mere proliferation, would fail to account for the growth   Totipotent or multipotent cell groups do not grow or develop unless isolated in a real though not necessarily physical sense from the rest of the organism   Otherwise they do not grow except as an orderly constituent of that organism, and when grafted elsewhere become resorbed   Growth of non-ovarian teratomas must accordingly be malignant growth from the very beginning   The problem therefore is that of the malignant change itself and of the peculiar teratomal development that follows   To a considerable extent it is a matter of emphasis

If a malignant change occurs in a relatively unspecialized cell one of several ways may be taken   Slight specialization invisibly expressed may result in spindle cell sarcomas, as from fibroblasts, and in the case of tumors of the testis seminomas arise probably from cells already too specialized to be multipotent   Cells entirely unspecialized may become actual rather than potential spermatogonia by virtue of their location   If such cells become malignant they may conceivably merely proliferate without other change of character, appearing as

semmoma types or even as so-called chorionepithelioma   On the other hand it is clear that malignancy so affects any cell that it behaves as if isolated from the organism   If multipotency or totipotency is innate in such a cell, and if all nutritive requirements for unlimited growth are met, as they are, the conditions for the expression of any independent developmental capacity are fulfilled

If is important to emphasize once more the dissociability of fundamental developmental phenomena, histogenesis, cell multiplication and growth, and morphogenesis (Needham, 1933)   Histogenesis may be considered by itself One of the major mysteries of development is the mechanism by which an indifferent cell gives rise to a progeny of diversely differentiated types   More is known of the induction of neurones from such cells and all that is certain here is that the inductor can be liberated from damaged or necrotic cells by an unrelated variety of agents   But the products of necrosis do call forth neurogenesis

In a growing mass of indifferent cells, among which many must be degenerating and dying, necrotic by-products must be plentiful and healthy cells of indifferent type may be induced to undergo initial segregations into a few simple types These may in turn interact or follow through their own respective destinies of further segregation   In any case the histogenetic situation is comparable to that of embryos and the problem of tissue segregation one and the same   Obviously the malignant quality does not inhibit the formation or existence of the various cell types

At the same time it is impossible to ignore the existence of certain rudiments of organization, and while organization itself requires explanation, even rudimentary organization may account for histogenetic segregation without calling on necrotic hormones

In advanced teratoma testis of the fowl feather follicles and glandular ducts are definitely organized structures, and it is evident that certain types of cells are able to cohere and undergo unitary development associated with their respective type   An experimental analysis of the general relationship between histodifferentiation and malignancy in teratomas is greatly needed   All that can be said is that the most specialized tissues proliferate slowest and are therefore the least malignant   But the non-transplantability of teratomas needs further investigation   It is possible that in the course of tissue segregation certain types are able to inhibit whatever underlies the malignant quality, and when proliferation is almost or entirely suppressed all the latent capacity for organization and differentiation within that mass of cells is expressed, the nature of the expression being determined by the degree of segregation previously attained

The other evidence of organization is found in the earliest stages of teratomal growth and in its essentials is amazingly similar in renal (Masson, 1938) testis and some ovarian tumors (Peyron, 1939)   Masson has given clear account of the development of nephrogenic tumors, while Peyron's descriptions, as distinct from his interpretations, demonstrate the essential similarity of testis teratoma development   According to Masson blastematous cords of the kidney gives rise to minute vesicles which he considers to be the sole and earliest tissue of origin

of the nephrogenic tumors   Each vesicle buds off other vesicles or cell masses which give rise progressively to a group of teratomatous tissues, including neurons, striated muscle and nephrons   He concludes boldly that the origin of the blastematous cords from the neuroepithelium of the embryo accounts for all the cell types encountered except muscle and nephron and that therefore in the embryo certain muscle and nephrogenic tissue must come from the mesectoderm of the neural crest and not from the mesendoderm, thus reversing the trend of interpretation from embryology to tumors!   It is implied that consequent on malignancy certain cells of neural crest origin progressively segregate into their prospective histological types   Their fate had already been somewhat determined so that multipotency persists, rather than the   tipotency of pros pective spermatogonia

The formation of vesicles that in turn bud off other vesicles and cell masses is common to both testis and kidney teratomas and must represent organization of a kind   Peyron considers those of testis to be blastocysts budding as in polyem bryony and giving rise to chorioplacental masses, embryomas, and much malignant chorioma stages and tissues plausibly named only if an origin from true parthenogenetic ova is valid   This cannot be established

In those forms, e g , ascidians, where a comparison can be made, there is a profound difference between the development of an egg and that of a group of totipotent indifferent cells (Berrill, 1941)   The egg cleaves to form a blastula, gastrula, tadpole larva etc , before transforming finally into a miniature adult The somatic cell group in contrast multiplies and forms a small vesicle which by extensions, foldings and subsequent histodifferentiation develops directly into a similar miniature adult without passing through any of the so-called historical phases of development

Blastocysts, embryonic discs fetal membranes belong to "historical" aspects of egg development, however essential they may actually be for normal development   There is no more reason to expect development from unspecialized cells in a mammal or bird to yield the early stages of egg development than in the lower chordates   It is more reasonable to compare the primary vesicles of teratomas with the primary vesicle of asexual ascidian development   Totipotent cells of higher vertebrate if able to develop at all might be expected to exhibit features comparable to the primary phases of bud development than to those of any developing egg

The repeated vesicular budding and final disintegrations may well be the result of malignancy   The following description of teratoma may accordingly be made

The malignant quality acquired by one or a group of indifferent totipotent cells of probable epithelial pedigree acts as an isolating factor in relation to the rest of the organism enabling it to exhibit whatever developmental potency it may possess   Proliferation begins, uncontrolled by the parent organism as in the case of malignant growth generally   Morphogenesis begins with the forma tion of a small vesicle   Either by virtue of its tissue environment or more likely because of the innate new quality of its component cells the system breaks down first by segregating daughter vesicles and cell masses and then by complete dis-

integration   The various cell strains initiated by this effort express the primary malignancy in various degrees, the least structurally specialized cells retaining the highest multiplication rate and therefore becoming the most malignant, such as epithelioma

The original malignant agent induces development and at the same time is responsible for its subsequent breakdown

Tumor induction   One of the most confusing aspects of malignancy is the diversity of the agents capable of inducing it, radium, x-ray, ultra-violet, polycyclic hydrocarbons, sex hormones, tumor extracts, butter yellow, arsenic and viruses We are faced with two alternatives   Either each kind of agent produces its own peculiar change in the cell and a large variety of qualitatively different changes enable cells to behave as though isolated from the parent organism, or, directly or indirectly, every such agent induces finally the same basic change in a cell, that is, a variety of stimuli call forth one and the same reaction   It is relevant to recall that many chemically and physically unrelated agents similarly evoke one and the same organizer response in amphibian embryos, all acting probably through the liberation of necrotic by-products

Both views are plausible   A variety of agents may in various ways induce the same qualitative change in a cell, uncontrolled growth being the result of the change in quality, or a variety of qualitative alterations produced by the various agents may call forth the same reaction if they exceed a certain magnitude

The malignant response and the proliferative response of normal cells to a disturbance of the condition of equilibrium as in regeneration and tissue culture must be considered together   Malignancy cannot be understood until much more is known concerning both cell division and its inhibition in tissues   Actual division of a cell is the climax of a complex process of growth and duplication of a multitude of cell components, while the inhibition of the process in vivo is clearly not due to lack of nutritive substances or to any deficiency on the part of the cell

Such evidence as there is suggests that normal cells refrain from or are prohibited from continuing division by surface conditions, by their relationship to one another   In a tissue they are under the control of the external agency to which they themselves probably contribute   Normal proliferation ceases when a condition of equilibrium is reached

There may be much more than an analogy between the induction of malignant behavior by chemical agents in tissue cells and the induction of divisions in eggs by parthenogenetic chemicals

Eggs admittedly are relatively giant cells and anything that restores to them the metabolism of normal tissue cells may be expected to start a series of divisions tending to restore typical somatic cell dimensions (Whittaker, 1933, Berrill, 1935)   But apart from this there are the significant facts that the most effective parthenogenetic agents are surface active compounds such as oxybutyric acid, that profound physical or physico-chemical changes occur immediately in the egg cortex, and that division asters are apparently induced *de novo* within the endoplasm

Since there appears to be a lower size limit to any given type of cell, all such cells must necessarily keep growing in order to continue to divide, whether under the impact of the regenerative stimulus or of a carcinogen   The question arises whether the cells are induced to continue growing and consequently keep dividing to maintain optimum dimensions or whether they are induced to divide and must grow to maintain the same surface-volume ratios

Tumor cells are generally described as being somewhat larger than their normal counterparts with large nucleoli (Lewis, 1938)   It may be significant that similar features characterize tissues preparing for or in process of regeneration (Sayles 1931)   Nuclei become vesiculate, nucleoli large and multiple, and cell size markedly increased, almost as though an overdrive is established

Lipoids, mitochondria, sex hormones, steroid carcinogens, avian tumor agents, and parthenogenetic chemicals are all surface active compounds and may be expected to affect primarily cortical or surface conditions of a cell   Any permanent abnormality in cortical regions may well so affect the intercellular relationships of a cell that it may be impossible to attain a state of equilibrium   Cortical cytoplasm and cortical relationships may be affected directly by external agents or indirectly by factors operating through their effect on nuclear constitution

Of the various tumor inducing agents, radium and x ray almost certainly primarily affect the nucleus   According to the dosage these produce either cell and nuclear destruction, permanent inhibition of mitotic division, or chromosome mutation   Induction of malignancy in somatic tissues occurs with much the same order of incidence and dosage as in the case of germ cell mutation   There is in this fact considerable support for Boveri's theory which so far has neither been proven nor disproven

Obvious chromosomal irregularity is probably incompatible with survival beyond a few cell generations, but slight irregularity compatible with continuing bipolar mitotic divisions is at least conceivable and yet such cells may be so changed that malignant behavior results   The more recent concept of somatic gene mutation does not differ basically from Boveri's chromosome theory   All that is required in either case is that some intra or inter-chromosomal change occurs that permits continuation of normal division processes and at the same time so changes a cell that it behaves as a physiologically isolated unit   It may be entirely a matter of chance whether any alteration of this type induced by x ray, radium or ultra violet leaves the division mechanism unimpaired and is of a kind expressing itself as malignancy

Colchicine apparently affects nuclear division in a non-differential manner, retarding or temporarily inhibiting the process during a critical phase   Since x rays seem absorbed and produce their dislocation effects during the more condensed chromosome phases, a combined use of colchicine and x ray should produce a much higher tumor incidence than the same x ray dosage alone   The negative result reported by Brues, Marble and Jackson (1940) does not appear to be conclusive

The chromosome aberration hypothesis, whether in the form of chromosome

dislocations or of gene mutations, raises the general question of the dependence of cell character on the persisting integrity of the whole nuclear constitution Much depends upon the interpretation of the amitosis phenomena   Until amitosis is demonstrable in living cells it is difficult to accept its existence as fully established   At the same time a pattern tends to emerge from the incidence of its reported occurrences   In all cases where it is established as a probability the cell types appear to be structurally relatively simple but highly specialized in chemical function   It is typical of proliferation in decidua and embryonic envelopes of vertebrates, the periblast of meroblastic ova, follicle cells in the ovary, endosperm, etc , of flowering plants (Belar, 1928)   It is described as the typical method of multiplication of bone cells (Bast, 1921), urodele erythrocytes (Charipper and Dawson, 1928), frog erythrocytes (Ferran, 1931) and of mature mammalian cartilage (Elliot, 1936)   It is suggested by Child (1907) and Elliot that it replaces mitosis when the demand for certain requisite metabolites such as oxygen exceeds the supply

Unless amitotic division masks unsuspected examples of endomixis and intra-nuclear bipolar segregation, it must result in qualitatively unequal division, and since typical cell character is maintained it follows that only a partial representation of full nuclear constitution is necessary for the continued amitotic division and special functioning of certain kinds of tissue   This is in contrast to the need for normal nuclear constitution for development and histogenesis and raises the whole question of the origin of cell differentiations and specializations and their maintenance   Obviously greater effort should be made to establish or disestablish amitosis as a fact, or to determine its true nature   If it is what it seems to be, and if chromosome aberration can lead to malignancy, tumors should frequently arise in the above mentioned tissues   They do not as a rule, though Butterworth (1937) suspects x-ray induced granulosa-cell tumors of mice to arise from nests of follicle cells after degeneration of ova

On the other hand there is considerable indirect support for the chromosome aberration hypothesis, which in any case is by no means an exclusive theory

According to Ishibasi and Harada (1933) the chromosome numbers in a series of human and animal tumors are in accord with the idea of chromosome disharmony being related to tumor development, though the question always recurs whether the relationship is a causal one or whether tumor formation and chromosome aberration are both products of some deeper disturbance

Carcinogenic cysticercus cysts induce irregular division and chromosome abnormalities almost immediately upon application and long before any other indication of malignancy can be detected (Mendelsohn, 1935)   Mitoses were found to be bipolar in spite of a much greater variability of chromosome number than in normal tissues

According to Huskins and Hearne (1936), the relatively high chiasmata frequency of the gonads of cancer-susceptible mice strains indicates a greater susceptibility in these strains to mitotic disturbance generally, that the processes of chromosome "splitting", spindle formation and cytokinesis are near the borderline of lack of correlation   From the study of maize, Jones (1940) con-

cludes that chromosome reallocations genes removed from some cells and replicated in others result in permanent alterations involving growth, color, size, shape and composition, that it is ' now evident why external agents such as physical radiations, carcinogenic chemicals, hormones viruses, parasitic organisms and what next can produce the same result as inherited tendencies, either by increasing the frequency of chromosome breaks and relocations or by altering the growth regulating regions in the chromosomes ''

Associated with chromosome aberrations is the widespread occurrence of multipolar mitoses in tumors As they are not invariably present in tumor tissue their presence has been considered by many to be a result of abnormal conditions and degeneration within the tumors, and that cells exhibiting such mitoses are not the malignant cells *per se* and have but a short life ahead of them This of course could be true without necessarily excluding multipolar mitosis as a causal factor

There are two distinct problems does the phenomenon precede malignancy or only follow it, and how is it to be interpreted in itself Only in the case of experimentally induced tumors is there much likelihood of determining whether multipolar mitosis precedes malignancy The evidence must inevitably remain ambiguous in the case of spontaneous tumors

The general effect of steroid carcinogens on growth of the whole animal appears to be retardative (Haddow and Robinson 1939) and the immediate effect on certain tissues at least is the induction of frequent multipolar mitoses Since there is on an average a latent period of several months between application and first detectable signs of tumor it is virtually impossible to consider such mitoses the product of malignancy Similarly a high incidence of multipolar mitoses was found to be a more or less immediate response to the presence of the Crocker cysticercus cysts, although here again there is a long latent period between cyst infection and tumor origin (Mendelsohn 1934)

There appears to be little doubt that in two outstanding types of tumor in ductors the tumors arise from tissues in which cell proliferation has continued for a relatively long period with varying degrees of abnormality Boveri's hypothesis is definitely supported by the facts and it is impossible to dismiss these abnormal mitotic phenomena as mere by products of established malignancy

It does not follow that tumors arise from the progeny of cells that have undergone multipolar mitosis although it is possible Multipolar mitosis may be an extreme case and other cells may react in other and less obvious ways Thus according to Hearne Creech (1939) in fibroblast cultures exposed to carcinogenic hydrocarbons chromosomes showed a precocious splitting in prophase, recalling the correlation between chiasmata frequency and mammary tumor susceptibility (Huskins and Hearne 1936)

In general carcinogenic hydrocarbons are growth inhibitors, non-carcinogenic hydrocarbons are not (Haddow and Robinson, 1939) Similarly there is a growth retardation and degeneration and lack of stimulation when mouse and rat fibroblast cultures are exposed to methylcholanthrene (Earle and Voegtlin 1938) Accordingly there is a probability that multipolar mitoses are more

likely to arise through the suppression of a cytoplasmic cleavage than from a precocious doubling of the centrosome, that they arise through differential retardation

Boveri's suggestion of lagging chromosomes with consequent deficiencies or reduplications in daughter cells lies somewhere between the multipolar condition and chiasmata formation   In the case of multipolar mitosis chromosome distribution to daughter cells is likely to be too abnormal for prolonged survival, and the two milder types of aberrant chromosome behavior are more likely to be compatible with the survival of proliferating cells   Bipolar mitosis is probably a requisite   The range of variability of chromosome number in tumors (Ishibashi and Harada, 1933) suggests lagging chromosomes and is hard to correlate with the types of segregation that would probably follow the multipolar condition

The fact that multipolar mitoses are so commonly a feature of malignant tissues has naturally led to the suggestion that malignancy is basically a disturbance of the centrosomal mechanism (Lewis, 1938)   In our present state of abysmal ignorance concerning the nature of the centrosomal force it is impossible to accept or to dismiss this hypothesis   Fogg and Warren (1940) found shortly after exposure of Walker rat tumor to x-ray that centrioles per cell increased to 4, 6 and 8 in up to 60 per cent of the cells, suggesting the predominance of one or more incomplete divisions, binucleate cells and multipolar mitoses being frequent   If there is a real disturbance of the centrosomal mechanism, it apparently relates to their activity in cytokinesis rather than their rate of reproduction

At the same time there may be a significant parallel between the induction of multipolar mitoses by carcinogenic hydrocarbons and the action of surface-active chemicals (cp Tyler, 1941) as inductors of accessory centrosomes in the eggs of various marine invertebrates

Orderly progressive division of nuclear constituents however is possible without spindle, plate, or breakdown of nuclear membrane, i e , endomixis (Painter and Reindrop, 1939) and may be followed by simultaneous division restoring the diploid condition (Berger, 1938)   There may also be an equivalent division of the centrosomes (Pollister, 1939)

As in the case of the vertebrate "organizer", the attractive hypothesis of chemical inductors of specific structural type has slowly yielded to a nonspecific conception   The cyclopentane ring and C20 methyl group typical of steroids and bile acids are shown to be unconnected with their carcinogenicity (Fieser, 1936)   According to Morton, Branch and Clapp (1936) one and only one structural similarity is evident, all have at least four benzene rings   Either four rings, or a single ring modified by its neighbors, must be present, for benzene as such has never been shown to produce cancer   Structure apparently cannot be the common factor relating the carcinogenicity of the various types of hydrocarbon, and maybe there is no common denominator   Fieser (1938) while more attracted to the idea of a definite chemical reaction states that there is no *a priori* reason for supposing that hydrocarbon carcinogenicity is not entirely

physical   Studies on the interaction of polycyclic hydrocarbons and steroids in surface films (Clowes, Davis and Krahl, 1939) indicate that interfacial forces may be strong enough to permit the hydrocarbons when present in animal tissues, to influence the physiological function of cholesterol and other sterols

In view of this, the fact that tumor cells have generally a higher lipoid content than most other types (Yasuda and Bloom, 1932) becomes highly significant, especially in view of the importance of lipoids as components of the surface layers and structural features of cells as well as in cell metabolism   Lipoids are a major constituent of mitochondria and, according to Grauffi (1939), carcinogenic hydro carbons have a selective affinity for these bodies

Accordingly these hydrocarbons conceivably may act as malignant agents in two ways   By so altering, qualitatively or quantitatively, the general steroid metabolism of the cell the processes of mitotic division may be differentially affected resulting in aberrant chromosome distribution in some degree, or by altering in some manner the steroid complex so that surface relationships are permanently changed

It may be relevant that steroids themselves, as estrogen, can induce malignancy in estrogen-sensitive tissue   It is possible, perhaps probable, that the induction is indirect, that overstimulation of the tissues results in an occasional chromosome mutation or reduplication of a type producing malignant behavior There is however evidence to the contrary   According to Gardiner (1936) male mice receiving small amounts of estrogen possess mammary glands resembling those of normal virgin females and develop very few mammary tumors, while those receiving larger doses show abnormal growth from the beginning and develop many mammary tumors   There is an implication that malignancy is the climax of growth abnormality   Loeb and his co-workers (1936) emphasize this view in connection with malignancy of the reproductive tract   In vagina and cervix, with increasing age, there is a lateral extension of surface epithelium leading to folds presumably under the influence of estrogens   Injection of estrogen intensifies and accelerates this process   The growth is not localized but is wide and of multiple areas   Step by step it passes to a precancerous (sic) condition and to beginning cancer   Very similar phenomena are reported for guinea pig peritoneum following estrogen injections (Lipschütz, 1942)   We have then this view, widely held by pathologists, that malignancy does not arise from a simple cell focus but is usually preceded by definite though vaguely described changes over a relatively wide area of tissue, and final malignancy may arise in multiple foci   This is supported by the effect of injections of cell free extract of lymphosarcoma in mice (Parsons, 1938) which produced mass changes in the lymphatic system

Self-synthesizing proteins of an alien type, as in the case of the infective papillomata virus of the wild rabbit, may stimulate growth locally   The majority of virus infections, while exhibiting a significant organ specificity, have no such effect and it may be that the particular protein type associated with rabbit papillomata affects surface relationships directly or indirectly whereas others do not   The heavy protein in this case stimulates infected epidermal

cells to abnormal growth and extracts of these induce similar growths in domestic rabbits (Kidd, 1938)   Extracts of these in turn are innocuous, and while inducing antibodies do not possess heavy protein in detectable quantity   It seems likely that in the domestic form the virus protein introduced in the extract is able to induce a proliferative response but is itself unable to reproduce in the domestic rabbit tissue   The pathogenicity of the wild type extract is destroyed by ultra-violet but not its ability to bind complement, indicating interference with its reproductive capacity   The occasional invasive malignancy of the papillomata may be properly compared with the induction of malignancy by estrogen   In both cases malignancy may be either the culmination of a progressive abnormality of growth, or it may be a distinct and more or less spontaneous malignant mutation in a favorably proliferating tissue

Nature of malignancy factor   Whatever the inductive agent may be it is probable that the final change in the cell directly responsible for malignant behavior is of a fairly specific kind   Cell proliferation is itself primarily a response to changing surface relationships and to place the onus of continuing division directly on a derangement of components of the mitotic mechanism such as the centrosome or to accuse genes regulating the normality of mitosis of misbehavior is to ignore the significance of size and growth

Cell multiplication implies protoplasmic growth except in the case of most eggs, enormous cells, with abnormal surface conditions, capable of subdivision into smaller and smaller cells until cortical and metabolic conditions are returned to normal   Tumor cells are no smaller and at the most are but little larger than their normal counterparts   Cell division must therefore occur when the cell size exceeds a certain critical value, else tumor cells should get progressively smaller and disappear   A cell divides primarily to retain certain volume-surface ratios of the whole and of its parts, and average cell size can be regarded as a form of equilibrium between essential surface activities and the mass of material   This applies not only to the cell as a whole but to all levels of its protoplasmic constitution   An alteration of surface conditions could affect the equilibrium so that it is reached at a different value and a larger or smaller average cell size might be established   According to Dodge (1937) methylcholanthrene has such an effect upon yeast cells

The factor for malignancy must accordingly satisfy the following requirements   It must either, directly or indirectly, so affect cellular interrelationships that cells are constantly endeavoring to attain a condition of tissue equilibrium that cannot be reached in the normal tissue environment with their new cortical constitution, or certain cellular components, if not all, continue to grow indefinitely and continued cell division is an inevitable response to increasing cell size   And it must be intimately associated, if not identical, with the agent determining the type of cell differentiation

Claude (1940) in an analysis of high speed centrifuge extracts of both avian tumor tissue and normal embryo finds particulate components of an average size of 70 millimicrons and of the general constitution of a phospholipid-ribonucleoprotein, therefore unlikely to be nuclear fragments   In the case of the chicken

sarcoma amounts of this sedimented material as small as 10^{-9} grams induce typical sarcomas upon injection   It has been stated that chicken tumors in duced by carcinogenic hydrocarbons could not be transmitted by cell free ex tracts (Mellanby, 1938) although non infective extracts injected into rabbits produce sera which neutralized filtrates of Rous sarcoma I (Fould, 1937)   Ex periments on a somewhat limited scale however show that there is no funda mental difference between the so-called chicken virus tumors and chemically induced tumors (Ames, Carr and Purdy 1939)   Dibenzanthracene added to hanging drop cultures of chick embryo fibroblasts and then infinitely diluted produced a culture that produced tumors upon injection, the particulate deposit from cell free extracts transmitting the tumor type readily   Similarly mouse sarcomas induced by dibenzanthracene and grafted for many generations have yielded cell free filtrates capable of transmitting the respective tumor types in an impressive number of cases (Parsons, 1936, 1938)

Claude's suggestion that mitochondria are involved in the malignant change is plausible   They are closely associated with structural differentiation of cells, are actively related to surface structure and properties, are intimately concerned with many aspects of cell metabolism, and are independent self perpetuating units   What little evidence there is suggests that they are concerned in the initial determination of cell character (Conklin, 1905, 1929)   They are disposed in a cell so as to supply a maximum surface so that the mitochondrial-cytoplasmic interface must be a significant architectural feature

The general rôle of cell division and multiplication relative to malignancy may have been overemphasized   In a limited sense it may be and probably is, basically important since the malignant cell appears to be radically and per manently different from its normal counterpart, and changes of all kinds are more apt to occur during the delicate and intricate procedure of division   Yet all the various peculiarities of cell multiplication and differentiation associated with malignancy may well be the consequences and not the cause of continuing protoplasmic growth   In a developing organism growing tissues may be under going rapid cell multiplication slow multiplication or no cell division at all, and yet the rate of growth is the same whatever the degree of cellulation and follows the same curve to end in a "steady state" applying to the whole (Berrill, 1941)   The fundamental problem is the nature of the agency creating such an end point and the course of growth leading to it   It is not a matter of nutrition   Malig nant tissue grows no faster than normal tissue can when explanted (Schrek 1936 Hoffmann Tenenbaum and Doljanski, 1939), and it is the failure to reach stability that is the crux of the problem   Whether continuing growth, auto catalytic or otherwise of a single component such as mitochondria involves growth of protoplasm as a whole is not known   Tumor metabolism is signif icantly different from that of normal tissues   According to Druckrey (1935) and Fleischmann (1936) it is the metabolism of injured tissues, while Dean Burk (1937) defines a tumor as a growing glycolyzing tissue with a deficient respiration   The problem seems to be infinitely complex and appears to be the nature of protein synthesis and reproduction   its relationships to cell surface

qualities and general metabolism, and the susceptibility of the whole to the inhibitory control of organization, whatever the basis of this may be

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PATHWAYS OF GLYCOLYSIS

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The last decade has witnessed tremendous progress in the understanding of the processes of carbohydrate metabolism   The combined efforts of many laboratories have resulted in the formulation of a fairly complete picture of the pathway of glycolysis in muscle extract and fermentation in yeast extract   Additional studies on many types of cells have suggested that there exists a common pathway widely distributed in nature for the formation of pyruvic acid from polysaccharides and hexoses   The pyruvic acid thus formed may be reduced to lactic acid, decarboxylated, or oxidized by a great variety of reactions which are only partially understood at the present time

The frequent occurrence of these reactions, leading to the formation of pyruvic acid, in nature raises the question as to whether this scheme represents the sole pathway for the formation of the key intermediate pyruvic acid   From time to time various workers have suggested that other pathways, particularly of a non-phosphorylating nature, are responsible for glycolysis in various tissues, especially tumor, brain and embryo

It is with these questions that this review will principally concern itself   Part one will consider the evidence for alternate pathways of glycolysis in animal tissues, and the question of the presence or absence of phosphorylation in such tissues   Relevant observations on the metabolism of other types of cells will be touched upon in part two

As a framework for the discussion, the Meyerhof-Embden-Parnas-Cori-Warburg cycle is presented in figure 1   This series of reactions, for purposes of brevity, will be referred to solely as the Meyerhof cycle throughout this review The reactions in figure 1 will not be discussed in detail since several recent reviews have covered this material (15, 16, 50)

Students of cellular metabolism are constantly faced with the fundamental difficulty of attempting to achieve understanding of *in vivo* reactions by the use of *in vitro* methods   Brei and tissue slice techniques have frequently been used to simulate physiological conditions   Such techniques have been employed to a very large extent in studies on alternate pathways of glycolysis   The use of breis and tissue slices, although possibly being more physiological than the use of extracts, introduces other complications   Thus such preparations do not allow free diffusion of added substrates, making quantitative or qualitative changes in the suspending medium difficult to interpret   In addition, the simultaneous occurrence of large numbers of reactions makes elucidation of pathways almost impossible   The alternative procedure has been to use tissue extracts and to attempt to study individual reactions by separating the components of such extracts   Such studies, when accompanied by adequate rate studies and tissue analyses, can at best give information as to which reactions can occur in intact tissue   The best understanding is obtained only by a comparison of the results

of both methods especially with respect to rates intermediate compounds and effects of inhibitors

GLYCOGEN + INORGANIC PHOSPHATE

‖ (phosphorylase)

GLUCOSE-1 PHOSPHATE

‖ (phosphoglucomutase)

GLUCOSE + ATP $\xrightarrow{\text{(hexokinase)}}$ GLUCOSE-6 PHOSPHATE

‖ (phosphohexosisomerase)

FRUCTOSE-6 PHOSPHATE

‖ (ATP + enzyme)

FRUCTOSE-1 6 DIPHOSPHATE

‖ (aldolase)

TRIOSEPHOSPHATE + INORGANIC PHOSPHATE

‖ (?)

1 3-GLYCERALDEHYDE DIPHOSPHATE

‖ (triosephosphate dehydrogenase + DPN)

1 3 DIPHOSPHOGLYCERIC ACID

‖ (enzyme + adenylic acid)

3 PHOSPHOGLYCERIC ACID

‖ (phosphoglyceromutase)

2-PHOSPHOGLYCERIC ACID

‖ (enolase)

2 PHOSPHOPYRUVIC ACID
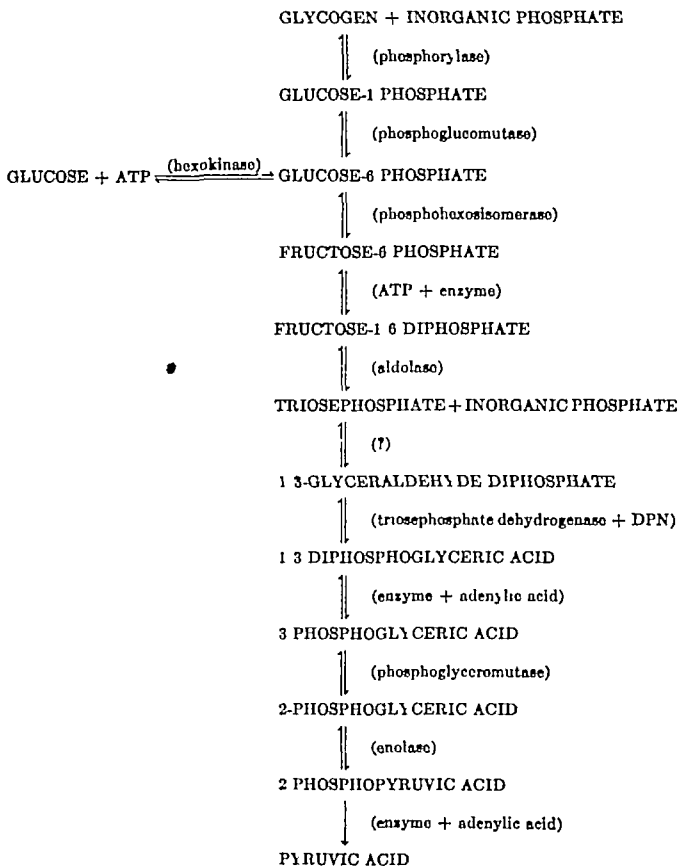
↓ (enzyme + adenylic acid)

PYRUVIC ACID

Fig 1 Meyerhof Embden Parnas-Cori Warburg cycle

Since certain types of experiments have been repeatedly performed to prove or disprove the occurrence of alternate pathways of metabolism, it seems wise to

analyze the literature in terms of types of evidence, rather than types of tissues
Each group of experiments will be analyzed according to the following criteria

1 Does the evidence prove the existence of the Meyerhof cycle?
2 If not, is it consistent with the Meyerhof cycle?
3 If not consistent with the Meyerhof cycle, does it disprove the existence of
   any type of phosphorylating cycle?
4 If a non-phosphorylating pathway is indicated, does the evidence indicate
   the nature of this pathway?

Selectivity of substrates   The difference in the relative rates of glycolysis of
various substrates by different tissues has attracted the attention of a number of
investigators   Such observations have been partially responsible for the postu-
lation of different pathways of metabolism

Needham and Nowiński (56) found that intact five day old chick embryos
produced lactic acid rapidly from glucose and mannose but were usually unable to
produce lactic acid from glucose-6-phosphate, hexosediphosphate, or glycogen
This work has been criticized by Macfarlane (40) on the basis that the results
were due to the fact that the phosphorylated sugars and glycogen were unable to
diffuse into the cells   Needham and Lehmann (58) have partially answered
these objections by showing that the added hexosediphosphate is broken down to
triosephosphate, and that this change occurs within the cells since the suspending
medium does not contain aldolase although extracts of embryo are rich in this
enzyme

Meyerhof and Perdigon (51, 52) have recently been able to prepare extracts of
both rat and chick embryos which are able to bring about the production of lactic
acid from hexosediphosphate plus pyruvic acid in the presence of fluoride, the
proper coenzymes, and a phosphorus acceptor   This finding leaves no doubt that
embryo tissue contains the essential triosephosphate dehydrogenase for the
production of lactic acid from hexosediphosphate

Needham et al (57), however, have been able to prepare breis which will bring
about the dismutation of hexosediphosphate and pyruvic acid only when adenyl
triphosphate (ATP), and diphosphopyridine nucleotide (DPN), are added, but
will actively glycolyze glucose without these additions   It would seem as if this
difference may be due to a lack of adequate phosphate acceptors in Needham's
preparations   Thus when hexosediphosphate is glycolyzed, the addition of
ATP results in its hydrolysis by ATP phosphatase to adenylic acid which can
then act as a phosphate acceptor and permit the necessary dephosphorylation of
phosphopyruvic acid   When glucose is being used, no added phosphate acceptor
is necessary since glucose itself acts as a phosphate acceptor   It would be of
interest to determine whether any phosphate acceptor could replace ATP in the
experiments of Needham

The failure of intact embryo to glycolyze glycogen is hardly significant since
this observation may be due either to lack of penetration into the cells, or absence
of the necessary phosphorylase for the breakdown of glycogen

The lack of rapid utilization of the phosphorylated hexoses and of glycogen
by brain presents a situation analogous to that in embryo   Thus the earlier

work by Ashford and Holmes (2) was done with brain brei, and is open to the criticism of permeability. Recently it has been possible to prepare extracts of brain (22, 23 64), which can utilize the phosphorylated hexoses as well as glucose Geiger (22, 23) showed that concentrated extracts of brain contain an inhibitor which prevents glycolysis, but on dilution such extracts readily convert glucose to lactic acid Geiger's work indicated that small amounts of hexosediphosphate increase the rate of formation of lactic acid from glucose and that phosphate is esterified during the course of glucose utilization Peculiarly enough, he found that no lactic acid formation occurred from the intermediate phosphorylated compounds of the Meyerhof cycle, although phosphate was required for lactic acid formation from glucose Ochoa (64), however was able to prepare extracts from brain which utilized the phosphorylated compounds and glucose at about the same rate The difference between the results of Ochoa and of Geiger may be due to the lack of phosphate acceptors in Geiger's preparations since Ochoa showed that addition of phosphate acceptors increased the rate of glycolysis of hexosediphosphate as well as the rate of dismutation between hexosediphosphate and pyruvic acid

Tzusuki (79), Matsuzaki (42), and Harrison and Mellanby (28) have all claimed that tumor tissue is unable to utilize phosphorylated hexoses at a rate comparable to glycolysis of glucose In contrast, Boyland and Boyland (12) have been able to show rapid glycolysis of phosphorylated intermediates by tumor extract

In summary it must be concluded from this group of experiments that tumor, brain, and embryo tissue contain the requisite enzymes for utilizing the phosphorylated hexoses which are intermediates of the Meyerhof cycle These experiments then indicate that the Meyerhof cycle can occur in these tissues and furnish no evidence to indicate that an alternate non phosphorylating pathway is instead operative

Summation Summation experiments have frequently been employed in determining whether two substrates are metabolized via the same pathway If two substrates are glycolyzed by the same enzyme system, then the total lactic acid production in the presence of optimal amounts of both substrates should not exceed that obtained from the compound used most rapidly However, if there is a difference in glycolytic pathways the lactic acid production in the presence of both substrates should be the sum of that obtained in the presence of each substrate separately It should be noted however that a difference in just one step in glycolysis of the two substrates is all that is necessary to produce evidence of summation providing that step is the slowest in the chain of reactions Thus, summation between glucose and glycogen does not indicate alternate pathways of glycolysis if one assumes the conversion of glycogen to glucose-6-phosphate (via Cori ester) or the phosphorylation of glucose to glucose-6 phosphate is the slowest step in glycolysis in any particular tissue

Ashford (3), using rabbit brain brei found summation of lactic acid production from glucose and glucose monophosphate and from glucose and hexosediphosphate while he found no summation from glucose and mannose On the basis

of these experiments he concluded that glucose and mannose are glycolyzed by the same set of enzymes while glucose and the phosphorylated hexoses are not converted to lactic acid through the same pathway   In these experiments glucose was used more rapidly than hexosediphosphate   The fact that the addition of hexosediphosphate, to a preparation glycolyzing glucose at a maximum rate, increases the lactic acid production, can readily be explained on the assumption that under these conditions the phosphorylation of glucose is the slowest of the series of reactions necessary for lactic acid production   The increase in rate of lactic acid production from hexosediphosphate, above that obtained from glucose alone, by the addition of glucose cannot be explained on the same basis However, the glucose may speed up the formation of lactic acid from hexosediphosphate by acting as a phosphate acceptor   As has been previously stated, Ochoa (64) and Meyerhof (51) have shown that the rate of dismutation of hexosediphosphate and pyruvic acid is increased by the addition of suitable phosphate acceptors necessary for the dephosphorylation of phosphopyruvic acid   Summation of lactic acid formation from glucose and hexosemonophosphate can be explained on the same basis if one assumes that the conversion of hexosemonophosphate to the diphosphate is a relatively slow reaction compared to the initial phosphorylation of glucose   An indication of the possible validity of such an explanation is that Ashford (3) obtained partial summation with hexosemonophosphate and hexosediphosphate   Thus it is possible that the results of Ashford can be accounted for within the framework of the Meyerhof cycle

Geiger (23) found no summation of lactic acid production from glucose and glycogen by diluted brain extract

B E Holmes (30) observed summation with glucose and hexosediphosphate using tumor brei, while Boyland, Boyland, and Greville (11) found no evidence of summation with glucose and hexosediphosphate using a tumor extract   The results of Holmes are similar to those of Ashford, the possible explanation of which has already been discussed   It is of interest to note that Boyland et al added adenylic acid, which is able to act as a phosphate acceptor   Holmes points out that the addition of ATP increases the rate of lactic acid production from hexosediphosphate but not from glucose   These observations are consistent with the interpretation that the glycolysis of hexosediphosphate requires the presence of a phosphate acceptor

Bumm and Fehrenbach (9, 10) claimed that a difference in glycolytic pathway exists between skeletal muscle and diaphragm   These workers, using a brei of skeletal muscle, observed no summation with glycogen and glucose plus hexokinase   However, with rat diaphragm glycolyzing the same substrates, summation was observed   They concluded that diaphragm, in contrast to skeletal muscle (red), has two different pathways for dealing with glucose and glycogen The results of these workers can be explained within the framework of the Meyerhof cycle if one assumes that the initial phosphorylations are the slowest steps in the process   The difference obtained between diaphragm and skeletal muscle may reflect a greater concentration of phosphorylase in the skeletal muscle

Although experiments on summation using brei do not provide positive evi-

dence for the exclusive operation of the Meyerhof cycle, they do not negate this possibility, and furnish no evidence for non phosphorylating glycolysis Experiments on extracts of brain and tumor support the interpretation that glycolysis of glucose proceeds through the same pathways as does glycolysis of the phosphorylated hexoses

Glyceraldehyde Much of the evidence in support of non phosphorylating glycolysis rests on conclusions drawn from the use of glyceraldehyde as an inhibitor Mendel (46) showed that *dl* glyceraldehyde at a concentration of 10^{-3} M inhibits almost completely lactic acid formation by tumor, but has little effect on respiration of tumor or normal tissue (liver, kidney, and brain) Mendel Bauch and Strelitz (47) found that the inhibition by 10^{-3} M glyceraldehyde is completely reversed by 10^{-3} M pyruvate and partially reversed by as little as $2\ 5 \times 10^{-5}$ M pyruvate Ashford (4) found that *dl*-glyceraldehyde also inhibited glucolysis In the same year E G Holmes (32) confirmed the work of Ashford, and also showed that the production of lactic acid by muscle from either starch or glucose plus hexokinase is not inhibited by this compound Needham and Lehmann (60) found that *dl* glyceraldehyde inhibited glucolysis and mannolysis of chick embryo about 90 per cent, and that this inhibition was reversed by pyruvic acid

On the basis of these results Needham (35), Ashford (4), and Tsusuki (79) have concluded that *dl* glyceraldehyde is a specific inhibitor for non phosphorylating glycolysis This view has been contested by Adler and co workers (1), Boyland and Boyland (12) Macfarlane (40), Süllmann (77), and more recently by Stickland (74)

The interpretation of much of the data on glyceraldehyde inhibitions is difficult Thus Boyland and Boyland (12), Süllmann (76), and Adler (1) claim that *dl* glyceraldehyde inhibits the glycolysis of glycogen, starch and glucose plus hexokinase by various tissues, while Needham and Lehmann (35 60), B E Holmes (30) and Baker (7) found no such inhibitions The following factors are possibly responsible for these discrepancies 1 Different concentrations of glyceraldehyde have been used by different workers 2 Lehmann and Needham (35) have shown that when *dl* glyceraldehyde is dissolved a dimeric form exists in solution This dimer inhibits the conversion of glycogen to Cori ester On standing, however, a dissociation to the monomeric form apparently occurs, and the solution no longer inhibits the phosphorolysis of glycogen but inhibits glucolysis by chick embryo This finding introduces a difficulty in assessing papers published before 1938 when this factor was recognized 3 Stickland (74) has recently shown that the degree of inhibition of muscle extract, glycolyzing glucose with the aid of yeast hexokinase is dependent upon the concentration of hexokinase He also found that the critical concentration of hexokinase varies with different extracts

Considering the factors listed above, certain general conclusions can be reached Glucolysis of brain, tumor and embryo preparations is inhibited by low concentrations of *dl* glyceraldehyde (below $0\ 01M$) The inhibition of glucolysis by muscle extract in the presence of yeast hexokinase is dependent on the concentra

tion of hexokinase   Inhibitions of glycogen and starch glycolysis require much larger concentrations of dl-glyceraldehyde and are probably due to the dimeric form

If lactic acid formation in embryo, brain and tumor tissues proceeds along the same pathway as does glycolysis in muscle, what underlies this marked difference in sensitivity toward glyceraldehyde?   Meyerhof et al (49) showed that the aldolase of muscle extract will bring about the irreversible condensation of d or l-glyceraldehyde with dihydroxyacetonephosphate to form l-fructose or l-sorbose phosphates respectively   These esters do not give rise to the equilibrium mixtures of hexose-6-phosphates   Macfarlane (40) suggests that in muscle and yeast the amount of dihydroxyacetonephosphate available for combination with glyceraldehyde is large, while in tumor, brain and embryo it is small   Thus, in muscle, the glyceraldehyde is readily removed and is unable to bring about inhibition, irrespective of mechanism of the action of glyceraldehyde   This view has been contested by Süllmann (77) and Stickland (74)   The former found that the rate of removal of glyceraldehyde from muscle extract glycolyzing glycogen is insufficient to account for lack of inhibition   Stickland pointed out that if Macfarlane's explanation were correct, then the more rapidly glycolysis proceeds, the greater should be the glyceraldehyde disappearance, with ultimate lifting of the inhibition   The opposite was found to be the case, i e , in muscle glucolysis proceeded at a much more rapid rate than starch glycolysis, nevertheless, the inhibition of glucolysis was much greater than the inhibition of lactic acid production from starch   A second objection raised by Stickland is that if the glyceraldehyde were being removed as glycolysis proceeds in the muscle extract, the inhibition should decrease with time, while in fact he found an increase with time

The work of Stickland (74), Süllmann (76, 77) and Adler (1) suggests that dl-glyceraldehyde inhibits the phosphorylation of glucose   Their observations also indicate an alternative explanation for the variation in tissue susceptibility to glyceraldehyde, for inhibition of glycolysis by glyceraldehyde is most striking in those tissues in which glucose is the principal substrate for glycolysis   This suggestion is attractive in that it provides a rather simple explanation for the observed differences between the two groups of tissues   The findings of Mendel (46) and Baker (7) present a possible objection to this interpretation   Both workers found that concentrations of glyceraldehyde which inhibited anaerobic glycolysis by tumor tissue did not affect the rate of oxidation of glucose by these tissues   It has generally been assumed that the oxidation of glucose by animal tissues is dependent upon preliminary phosphorylation   If glyceraldehyde inhibits this initial phosphorylation, inhibition of respiration should parallel inhibition of glycolysis   Three possible explanations of this discrepancy suggest themselves   The first is that aerobic phosphorylation of glucose may occur through a different mechanism than does anaerobic phosphorylation   It is interesting to note that Loebel (38) and Dickens and Greville (17, 18) found that fructose could be rapidly oxidized by brain brei, but is not glycolyzed anaerobically   Gerard and Meyerhof (25) made a similar observation on nerve   A second

possibility is that glyceraldehyde inhibits the phosphorylation of glucose-6-phosphate to hexosediphosphate rather than the initial phosphorylation of glucose The latter suggestion is made less likely by the fact that Stickland (74) obtained reversal by a hexokinase preparation, which presumably esterifies glucose to glucose-6-phosphate Another objection to this interpretation is that Süllmann (77) found no inhibition of lactic acid formation from glucose monophosphate A third possibility is that under aerobic conditions a reaction occurs which results in the removal of dl-glyceraldehyde

Any explanation of the mechanism of glyceraldehyde inhibition must explain reversal by pyruvic acid Mendel et al (47), using tumor brei, found that pyruvic acid reversed glyceraldehyde inhibition of glucolysis. Holmes (32) observed no pyruvic acid reversal with brain slices Baker (7) confirmed the results of Mendel with tumor tissue, observing partial reversal of glyceraldehyde inhibition when the glyceraldehyde inhibition was complete, and more complete reversal at lower glyceraldehyde concentrations She found as did Holmes, no reversal of the inhibition of brain glycolysis Needham et al (60) noted that the glyceraldehyde inhibition of embryo glycolysis could be partially reversed by pyruvic acid

The mechanism by which pyruvic acid reverses glyceraldehyde inhibition has received no adequate explanation It is known that glyceraldehyde in large concentrations reacts with pyruvic acid (through the mediation of the appropriate enzyme and coenzyme), in the same manner as does glyceraldehydephosphate This reaction can remove glyceraldehyde, irrespective of the mechanism of glyceraldehyde inhibition Süllmann (77) found that the inhibition of glycogen glycolysis by muscle extract by 0 1 M glyceraldehyde could be reversed by 0 022 M pyruvate, with lactic acid production This is consistent with the above explanation However Stickland (74) was unable to find any evidence of reaction between glyceraldehyde and pyruvic acid, when both reactants were present in a concentration of 0 003 M

Another possible explanation for the reversal by pyruvic acid depends on the interpretation that glyceraldehyde inhibits metabolism by inhibiting phosphorylation It is possible that the phosphorylation in tumor and embryo is linked with the reoxidation of reduced diphosphopyridine nucleotide In such a case the presence of pyruvic acid might serve to reoxidize the reduced diphospho pyridine nucleotide and directly or indirectly to overcome the inhibition It is of interest to note that Baker (7) found that aerobic glycolysis is more resistant to glyceraldehyde inhibition than is anaerobic glycolysis The lack of reversal in brain, observed by both Baker and E G Holmes, may be due to a difference in mechanism of phosphorylation in brain

By the use of partially resolved glyceraldehyde, Needham and Lehmann (60) showed that the l-glyceraldehyde is responsible for inhibition of embryo glycolysis This observation has been confirmed by Mendel, Strelitz and Mundell (48) working with tumor tissue Süllmann (77), working with lens extracts, found the d and l forms equally active He worked, however, with much larger concentrations of glyceraldehyde

Needham and Lehmann (60) also showed that the d-form is changed non-enzymatically to methylglyoxal, which is converted to lactic acid by methylglyoxalase   This observation is of interest in view of the fact that most workers have reported that glyceraldehyde inhibition is not complete even at high concentrations   Such results are probably due to the formation of lactic acid from the glyceraldehyde   Baker (7) observed a stimulation of glycolysis in liver and kidney which may also be due to lactic acid production from d-glyceraldehyde Stickland (74) noted a similar increased CO_2 liberation from liver in the presence of glyceraldehyde, but found that only a small part of this was actually due to lactic acid, determined chemically   He attributed the manometric results to the formation of glyceric acid by the aldehyde mutase in liver

The literature of glyceraldehyde inhibition has been reviewed in considerable detail since many workers have regarded such evidence as of paramount importance in proving that glycolysis in certain tissues does not proceed through the pathway demonstrated for muscle extract   At this time it is not possible to conclude that the work on glyceraldehyde inhibition provides positive evidence for the existence of the Meyerhof cycle in the tissues under discussion, but such experiments do not necessitate the postulation of alternate pathways   It is obvious that, aside from certain minor discrepancies, the inhibition of glucolysis by glyceraldehyde is consistent with lactic acid production through phosphorylated intermediates   In no case is it possible to conclude that the work on glyceraldehyde provides positive evidence for the existence of a non-phosphorylating pathway

Fluoride   Sodium fluoride has been frequently used in studies on the mechanism of glycolysis and fermentation   The earlier literature on fluoride inhibition has been reviewed by Gemmill (24)   The mechanism of fluoride inhibition has recently been elucidated by the studies of Warburg and Christian (81) These workers have separated enolase (80), the enzyme responsible for the conversion of 2-phosphoglyceric acid to 2-phosphopyruvic acid, and found that its active form exists either as the zinc, manganese or magnesium salt   The zinc enolase is inhibited by hydrocyanic acid, while the magnesium enolase is very sensitive to fluoride   The zinc and manganese salts are also inhibited by fluoride, but at considerably higher concentrations   The degree of inhibition of the magnesium enzyme was found to be dependent on the relative concentrations of magnesium, phosphate, and fluoride

Differences in sensitivity to fluoride have been used by some workers as evidence to prove the existence of either non-phosphorylating glycolysis, or at least the existence of alternate pathways of glycolysis   Thus Ashford and Holmes (2) found that formation of lactic acid from glycogen and from glucose by brain brei differ in sensitivity to fluoride   They also found that fluoride did not inhibit the appearance of free phosphate to the extent expected when glucose was used as substrate   A possible explanation of these effects is that fluoride indirectly inhibits the phosphorylation of glucose by brain as a consequence of its effect on enolase   Thus, if the enolase reaction is stopped, phosphopyruvic acid is not formed and ATP cannot be resynthesized, resulting in the inhibition of

glucose esterification This interpretation is supported by the work of Ostern, Baranowski and Terszakowec (65), who found that sodium fluoride inhibited the formation of adenosine triphosphate in yeast extract from adenosine and phosphoglyceric acid

Scharles, Baker and Salter (72) found that 0 05 M fluoride did not affect lactic acid formation from hexosemonophosphate by tumor extracts Since much lower concentrations of fluoride (0 001–0 005 M) generally inhibit glycolysis by muscle extract these workers consider this observation as indicative of a different pathway of metabolism In view of the finding of Warburg and Christian that the sensitivity of enolase is dependent on the magnesium and phosphate concentration, it is difficult to rule out the validity of the Meyerhof cycle in tumor tissue on the basis of differences in fluoride sensitivity

Scharles *et al* (72) also found that their tumor extracts were able to convert hexosephosphates to lactic acid, with no sensitivity to fluoride at 52° It is likely, however, that in this case the results were due to non-enzymic conversion of triosephosphate to methylglyoxal which was in turn converted to lactic acid by methylglyoxalase It is doubtful whether such a pathway is of any physiological significance

Kerly and Bourne (34), using retinal extracts, found that smaller concentrations of fluoride are required for the inhibition of glucolysis than are required for lactic acid formation from hexosediphosphate The explanation of the results of Ashford and Holmes would seem to apply equally well to those of Kerly and Bourne

Needham *et al* (57) failed to observe the expected accumulation of phosphate esters during the glucolysis of embryos poisoned with fluoride, although an increase in the hexosediphosphate fraction is evident in some of their data. Somewhat similar observations were made by B E Holmes (31), who noted that 0 001 M fluoride inhibited the formation of lactic acid from glucose, but that no esterification of phosphate occurred These results again are consistent with the Meyerhof cycle if one considers the possibility of indirect inhibition of phosphorylation by fluoride

Needham *et al* (57) have claimed that with a chick embryo preparation inhibition of glucolysis did not occur until a concentration of fluoride of $M/50$ was reached, while with the same preparation $M/200$ fluoride completely inhibited the enolase reaction (phosphoglyceric→phosphopyruvic) Macfarlane (40) has suggested that this finding may be due to accessibility of the enolase at the cut surface to both the phosphoglycerate and the fluoride. However, when glucose is used as substrate, the cells must be penetrated by fluoride for inhibition The validity of such an interpretation lacks experimental support The observation of Needham is unusual in that other workers have usually found glucolysis to be more sensitive to fluoride than the glycolysis of phosphorylated intermediates (Ashford and Holmes (2), B E Holmes (31), Kerly and Bourne (34))

Werkman and co-workers (83, 86) have shown that *Propionibacterium pentosaceum* can be grown on a medium containing large concentrations of sodium fluoride, and retain the ability to ferment glucose while becoming insensitive to

fluoride Such organisms are unable to ferment phosphoglyceric acid The parent strain is able to ferment both glucose and phosphoglyceric acid and both fermentations are inhibited by fluoride This work suggests that the "trained organisms" are able to ferment glucose through mechanisms not involving phosphoglyceric acid Similar results have been reported by Stone *et al* (75) for *Aerobacter aerogenes*

On the whole, the work on fluoride gives little reason to doubt the existence of the Meyerhof cycle in mammalian tissues The observations of Needham, which cannot be obviously explained within the framework of the accepted cycle lack confirmation The observations in any case do not prove the existence of a non-phosphorylating pathway

The work of Werkman and co-workers indicates that, in micro-organisms, alternate pathways do exist, but again, there is no evidence in support of a non-phosphorylating pathway No such claim has been made by the authors

Ashford and Holmes (2), studying the effect on glycolysis of removing free phosphate by adding an excess of calcium chloride to rabbit brain brei, found that such treatment inhibited the glycolysis of glycogen but not that of glucose It is hardly likely that complete removal of phosphate could be achieved by such treatment, but a decrease in phosphate concentration did occur Addition of phosphate restored acid production from the glycogen A possible explanation of these results without contradiction of the Meyerhof cycle depends upon the fact that the phosphorylation of glycogen is dependent upon the concentration of inorganic phosphate (14), while the phosphorylation of glucose is accomplished with phosphate derived from ATP Thus a decrease in the concentration of inorganic phosphate would affect glycolysis of glycogen to a much more marked extent than that of glucose

Needham (59), performing similar experiments on embryo brei, found that excess calcium chloride had no effect on glucolysis The formation of lactic acid from glycogen was not studied No analytical evidence for the complete removal of phosphate is given Macfarlane (40) and Macfarlane and Weil-Malherbe (41) have recently pointed out the extremely small quantities of phosphate that are required for glycolysis in both intact yeast and brain slices The phosphate is continually regenerated and thus acts in catalytic quantities They have also shown the extreme difficulty involved in the complete removal of phosphate The literature to date contains no conclusive demonstration of mammalian glycolysis in the absence of phosphate

Several workers have attempted to prove the absence of the Meyerhof cycle by studies of the ability of the various tissues to bring about the successive steps of the cycle Many older studies are of little value since they do not consider recent modifications of the cycle

The most detailed study along these lines has been that of Needham and Lehmann (58) on chick embryo They found that embryo possessed all of the necessary enzymes with the exception of the triosephosphate dehydrogenase Similar claims have been made by Tsuzuki (79) concerning tumor tissue However, Meyerhof and Perdigon (51, 52) have recently been able to demonstrate the

presence of this enzyme in adequate amounts in extracts of both chick and rat embryos Similarly the presence of the necessary enzymes has been demonstrated in brain (64), tumor (11, 12) and retina (34)

From these observations it would seem that, whether or not the principal pathway of glycolysis in these tissues is through the known phosphorylated intermediates the necessary enzymes for this pathway can be extracted from these tissues Thus the Meyerhof cycle cannot be ruled out in the tissues under discussion on the basis of lack of the necessary enzymes

The possibility of oxidation of carbohydrate without previous phosphorylation is suggested by the work of Harrison (27) and of Hawthorne and Harrison (29) on the glucose dehydrogenase of liver This author has been able to extract an enzyme from beef liver which brings about the oxidation of glucose to gluconic acid It apparently is able to function with either di or triphosphopyridine nucleotide as coenzyme From the work of Harrison and of Rice and Dorfman (67), this enzyme does not appear to be identical with the hexosemonophosphate dehydrogenase of Warburg The importance of this enzyme in carbohydrate metabolism remains to be determined

Much has been written of the effect of the structural organization of the cell upon the pathway of metabolic reactions, but as yet little experimental evidence is available along these lines This subject has been discussed in a recent review by Commoner (13) Of particular interest is the observation of Gaddie and Stewart (20) on intact heart muscle. These workers found that, on repeated contraction, the carbohydrate stores could be depleted The addition of certain substrates restored contraction Among these was found methylglyoxal This observation is of interest in that methylglyoxal can be both produced (21) and used by muscle (20, 39) and suggests that this compound may be capable of furnishing energy for contraction

The question as to whether the Meyerhof cycle is operative in normal muscular contraction has been considered by Sacks (68, 69), and has been reviewed by Sacks (70), Meyerhof (50, 53) and Cori (15, 16)

As in the case of mammalian tissues there has been disagreement as to the mechanism of fermentation in microörganisms The fact that a phosphorylating mechanism does exist in many microörganisms has been amply demonstrated by a number of observers particularly Werkman and co-workers (82) However, certain observations have raised doubts as to the general application of the Meyerhof cycle in all microörganisms

Fisher (19) stated in 1895 that fermentation of disaccharides or polysaccharides always involves preliminary hydrolysis Willstätter and Oppenheimer (85) and Willstätter and Steibelt (84) challenged this view when they found that certain yeasts were able to ferment lactose and maltose more rapidly than the constituent monosaccharides. They furthermore observed that the fermentation of the disaccharides is not correlated with the content of the appropriate hydrolytic enzymes of the particular yeast Similar results were obtained by Sobotka and Holzman (73) Wright (87 88 89) has more recently observed similar phenomena with respect to the fermentation of sucrose and lactose by

Streptococcus thermophilus, while O'Connor and Nord and Engel (63, 62) have suggested that *Fusarium lini* Bolley ferments trehalose directly   Leibovitz and Hestrin (36) have investigated this problem by comparing the rate at which maltose and methyl-α-glucoside are fermented by yeast and have come to the conclusion that maltose is fermented directly

The mechanism of this phenomenon has not been satisfactorily explained No phosphorylated forms of the disaccharides mentioned above have been isolated   It would seem, however, that a possible explanation of these data would be the direct conversion of the disaccharides to two molecules of hexosemonophosphate, in a manner analogous to the phosphorolysis of glycogen by phosphorylase   The hexosemonophosphate could then be broken down by the usual series of reactions   A reinvestigation of this problem from such a point of view should merit consideration   This would be in accord with the findings of Baba (5, 6) that phosphoglyceric acid is an intermediate in the fermentation of maltose by yeast

The presence of enzymes in moulds capable of metabolizing carbohydrates without phosphorylation seems likely   Examples can be found in the work of Müller (54, 55) on glucose oxidase of *Aspergillus niger*, the formation of citric acid by *Aspergillus niger* (33), and the production of kojic acid by *Aspergillus tamarii* Kita (26)

Additional information on mould metabolism has been covered in the reviews by Tamiya (78), Raistrick (66), and Benhauer (8)

An attempt has been made critically to evaluate the evidence for the existence, particularly in animal tissues, of pathways of carbohydrate metabolism other than the Meyerhof cycle   The Meyerhof cycle is the result of numerous studies on extracts of muscle and yeast   The analysis of the reactions of the cell has necessarily been approached by a study of the presence and properties of enzymes that can be obtained from the disrupted cell   This has been done in most cases with a full realization of the limitations of such studies

Studies of energy relationships, the action of inhibitors, rates of reactions, and the extractable enzyme components of tissues present a picture of possible reactions within the cell, but lead to only probable conclusions as to the course of these reactions within the cell   Since, however, a tremendous weight of evidence has accumulated favoring the existence of the Meyerhof cycle, the likelihood of its component reactions playing some important part in the degradation of carbohydrates within the cell seems very great   Therefore it becomes essential to determine whether any evidence is available to indicate that this series of reactions is not of primary importance within the cell   On the basis of the analysis presented in this paper there is at the present time little evidence pertinent to animal tissues which requires the assumption of a radically different scheme of glycolysis   The evidence for non-phosphorylating glycolysis is even less convincing

In the case of microörganisms, suggestions of alternate pathways of glycolysis have a somewhat greater experimental basis

The explanations offered in the review have for the most part depended on the

recent work of Meyerhof and Ochoa on the necessity of phosphate acceptors, the work of Cori and co-workers on mechanisms of phosphorylation, the work of Warburg and Christian on enolase and fluoride inhibition, and the work of Stickland on glyceraldehyde   It should be remembered that such explanations are suggested to indicate that the evidence does not require the acceptance of a new series of reactions to explain glycolysis in tumor, brain, and embryo   In many cases considerably more experimental data are required to prove the validity of such interpretations

Thus the evidence at present does not rule out the possibility of the existence in animal tissues of glycolytic pathways other than the Meyerhof cycle, but the postulation of such alternate pathways is at present unnecessary to explain the facts

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INFLUENCE OF ESTROGENS AND ANDROGENS
ON THE SKELETAL SYSTEM

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Sexual differences in the extent and form of osseous growth have been noted in many species, and in some species cyclic changes in the gross and microscopic structure of at least parts of the bones have been found to be associated with reproductive phenomena This review will deal with the nature and extent of the influence of gonadal hormones on skeletal development and the studies pertaining to the mechanisms governing these changes. In connection with the latter studies it will also be necessary to consider the influence of sex hormones on the metabolism of bone salts The great difference in the responsiveness of animals of different species has made it desirable to present this review more or less in phylogenetic arrangement

INFLUENCE OF SEX HORMONES ON CALCIUM AND PHOSPHORUS METABOLISM
The changes in the levels of blood calcium and phosphorus which accompany egg laying and estrogen production have recently been reviewed (1) However, since many of these changes also affect the skeletal system they will be reviewed here to the extent to which they bear a relationship to, or afford an explanation of, the mechanism governing those osseous changes which are under the in fluence of the sex hormones

Calcium metabolism—fish and amphibia Hess et al (2) showed that during the period when the eggs develop and mature in females of the cod and puffer fishes, the blood calcium level rises to 29 mgm per cent as compared to 9 to 12 5 mgm per cent in males In the viviparous dogfish shark, on the other hand, no change in calcium is associated with the reproductive state Again, in the toad Xenopus (3) the level of calcium in females is higher during the egg laying season than during the non breeding season and at all times is higher than in males Castration effectively lowers the calcium values in females and, after a prolonged period, in males Ovarian extracts cause a rise in the blood calcium level in castrated or hypophysectomized females. Unfortunately the skeleton has not been studied It was thought that the hormone-like action on calcium is exerted by some principle other than the sex hormones (3, 4) However, more recent investigation on other species has indicated that estrogen is the responsible hormone

Aves The most significant observations on calcium metabolism have been made on birds, as would be expected, since enormous amounts of calcium are excreted in the egg shell (5 5 grams of calcium carbonate in 16 hrs or 40 mgm. of calcium per min during certain periods while the shell is being laid down) (5) The pigeon is an excellent animal on which to study the correlation between increased serum calcium levels and egg laying (6) since the nature of its laying habits makes it possible to predict with accuracy the stage of development of the ovum at any given time Two egg yolks are ovulated and then the ovary enters

upon a prolonged inactive period   During the period of rapid growth and ovulation of the two ova the serum calcium increases from 9 3 mgm per cent, the value for sexually inactive females, to 19 9 mgm per cent   Since the calcium level begins to rise 123 hours before the secretion of the first egg shell, it is obvious that the increase in serum calcium is not caused or explained by shell secretion The rise is correlated only with the development of the two ova   Similar differences also occur between the serum calcium levels of non-laying and laying hens (7, 8) and are correlated with ovum size (9, 10, 11)   Attempts have been made to show that the fluctuations which occur in the laying hens are due to a mobilization of calcium in the blood stream when an egg is not in the shell gland and a lowering of the serum calcium level when the egg is in the shell gland (5, 12), but the variations found were too small and inconsistent in trend to be very significant (13, 14)   However, evidence, acquired by determining the ratio of CaO to P_2O_5 stored and excreted, indicates that calcium can be deposited in bone and from this reservoir be mobilized for formation of the egg shell (15, 16, 17)   The amount that can be mobilized has been variously estimated as from 10 per cent (18) to 25 per cent (17) of the total calcium stores of the body   Mobilization of calcium from the skeleton is rapid but not as rapid as is the transference of ingested calcium carbonate to the oviducts (17)   Therefore, if sufficient amounts of calcium carbonate are ingested, the shell gland is supplied from this source (19, 20), but if the calcium carbonate intake is low, calcium is withdrawn from the bones (15, 17)   In the pigeon, however, it has been shown by careful histological study (21) that there is some destruction of medullary bone at the time of egg shell formation even though sufficient amounts of calcium are available in the diet

In the non-laying hen the mesenteric vein contains the same level of serum calcium as does the mesenteric artery, but in laying hens the calcium in the vein is appreciably greater than in the artery (19)   This increase in the amount of calcium transferred from the gut to the blood stream is evidently controlled by estrogen since it occurs only during the period of ovarian growth and egg laying If calcium is withheld from the diet of the laying hen, egg laying ceases after about 12 days and the amount of calcium in the egg shells decreases to less than one-fourth the normal amount, indicating that only a limited amount of calcium can be withdrawn from the body stores (bones) for the production of shells (13) It has already been shown that the calcium brought by the blood to the oviduct is in a form which is different from that deposited in the egg shell (22)   The extreme fluctuations in the serum calcium level which take place in the bird can be tolerated because the diffusible calcium remains constant, and only bound or non-diffusible calcium increases (23, 24, 25, 26)   This hypercalcemia bears no relationship to the hypercalcemia observed in mammals which results from hyperparathyroidism or administation of parathyroid extract (27)   Parathyroid extract does not cause a rise in blood calcium or a depletion of body calcium in non-laying hens, moulting hens, immature pullets, or cockerels (28, 29)

Influence of different estrogenic chemicals   Theelin, theelol, estradiol and estradiol benzoate increase the serum calcium level in pigeons (27, 30, 31), and

theelin, estradiol, estradiol benzoate and diethylstilbestrol are effective in this respect in chickens (32, 33, 34, 35)   Likewise estradiol benzoate is effective in the duck (36) and the sparrow (37)   Only Marlow and Koch (38) and Marlow and Richert (39) have reported negative results from the injection of estrogenic compounds into chickens, but it seems probable that they were using inadequate amounts of estrogen, since the doses they employed are too low to induce complete development of the oviducts.

Phosphorus metabolism   A great rise in blood lipid phosphorus levels occurs in apparently all oviparous vertebrates during the breeding season or following estrogen injection   This rise is presumably associated with the formation of the egg yolk   There is also a phosphoprotein (serum vitellin) which comprises an important part of the increased total phosphorus in breeding females of birds (40, 41, 42, 43), reptiles and fishes (40), but is found only in traces, if at all, in males and non breeding females of these species   Unfortunately amphibia were not tested   Serum inorganic phosphorus, the only phosphorus compound associated with the metabolism of bone, has been followed rather incidentally to the studies on calcium and bone changes.   It seems to be relatively less affected by the reproductive state of the animal or by sex hormones than are the lipid and protein phosphorus compounds   However, the intimate relationship of the serum inorganic phosphorus to the latter compounds makes interpretation difficult

Amphibia   In the lower vertebrates inorganic phosphorus has been studied only in the toad Xenopus where, in contrast to calcium, it is decreased during the breeding season, and after injection of progesterone and estradiol benzoate (44, 45)   However, the same author (44) reports that gonadectomy has no effect on plasma inorganic phosphorus

Aves   In connection with the studies on the relation of calcium metabolism to egg shell formation in chickens it was noted that more P_2O_5 was excreted than ingested, indicating that calcium salts were being removed from bone for egg shell formation (15), and that the associated phosphorus was being released into the blood stream and excreted   This condition is similar to that found in hyperparathyroidism, but it is doubtful whether such a calcium phosphorus relationship is also the explanation of the increase in serum inorganic phosphorus which occurs during the time bone is being deposited under the influence of injected estrogen   Small, probably inadequate, doses of estrogenic extracts have been reported to be ineffective in altering the serum inorganic phosphorus of chickens (38), but larger doses of estrogens raise the inorganic phosphorus in chickens (32), in ducks (36) and in pigeons (27)

Phosphatase activity   The serum phosphatase value in chickens (46) increases from 88 7 Bodansky units at one day of age to 114 0 at 10 or 12 days   It then gradually falls without showing any sex differences until the approach of the adult condition   In pullets the serum phosphatase value is 3 5 Bodansky units while in older non-laying hens it is 13 1 and in laying hens 17 1   In the mature cock the serum phosphatase value remains at 4 7   The relation of serum phosphatase to injected steroid hormones has not been studied

INFLUENCE OF SEX HORMONES ON SKELETAL STRUCTURE IN BIRDS   *Pigeons*
During the course of investigations on bone marrow Kyes and Potter (47) dis-
covered that at certain times the marrow cavities of female pigeons became so
filled with spicules of bone that it was no longer possible to obtain marrow from
them, but this phenomenon was never encountered in male pigeons   These
authors further observed that the condition of the bones in the females was cor-
related with the size of the ovarian follicles   When the follicles were 2 mm in
diameter, or less, the femurs contained large marrow spaces   Osseous tissue
partially invaded the marrow when the follicles were more than 4 5 mm in
diameter, and the marrow cavities were filled with bony spicules when the follicles
were 10 0 mm in diameter, or larger   Shortly afterwards Riddle and Dotti
(30) demonstrated for the first time that injected estrogens cause a rise in serum
calcium such as occurs normally at the time the ovary contains large ova   It
was then but a short step to show that both hyperossification and the increase in
blood serum calcium in pigeons can be produced by injected estrogen (31)   It
was found that with daily injections of 33 3 μg of estradiol benzoate hyperossi-
fication began within two weeks after treatment was started and reached a max-
imum within five weeks, but it did not exceed that found in the normal female
pigeon just after ovulation   Bloom, Bloom and McLean (21) made a careful
study of the histology of the bone changes which occur during the normal egg
laying cycle of the pigeon and found a very close correlation between the size of
the ovarian follicles and medullary bone formation   An intense production of
medullary bone occurs during the time of development and maturation of the
follicle in the ovary and is followed by a period of intense destruction of medullary
bone which coincides with the time the shell of the first egg is being formed, and
continues for a few hours after the first egg is laid   Bone formation then begins
once more and continues until shortly after the second egg reaches the shell
gland   Again bone is resorbed while the second egg is in the shell gland and
resorption continues at a diminished rate after the second egg is laid until all of
the medullary bone disappears (about 10 days after the second egg is laid)   The
pigeon lays only 2 eggs in a clutch   During the bone formation phases, the
reticular cells of the marrow become osteoblasts and these in turn become osteo-
cytes   During the destruction phases the osteoblasts and freed osteocytes be-
come osteoclasts, which, during the period of bone formation occurring between
the laying of the two eggs, may again become osteoblasts   During the final
postovulation period both osteoblasts and osteoclasts become reticular cells once
more   With an adequate intake of calcium and phosphorus, formation of the
organic bone matrix and calcification occur simultaneously, and during the
periods of rapid bone destruction both are removed simultaneously   The bone
formation associated with follicular development takes place in the granulocyto-
poietic portions of the marrow   Blood vessels are not encroached upon, and
erythropoiesis is not appreciably affected   However, myelocytopoiesis practic-
ally disappears from the marrow at this time
     It was fortunate that Pfeiffer and Gardner (31) used sexually active male
pigeons in their experiments on the relation of estrogen to hyperossification, since

Bloom, McLean and Bloom (27) discovered that doses of estradiol sufficient to induce medullary bone formation in male pigeons during the breeding season are ineffective in castrates or in males with inactive testes   However, the simultaneous administration of testosterone propionate and estradiol caused the formation of medullary bone with as little as 5 μg of estradiol per day   The maximum effect of testosterone propionate in the combined treatment was reached with 1 0 mgm. daily   This synergism between male hormone and estrogen suggests a possible function for the appreciable amounts of male hormone produced by the ovaries of birds, studied particularly in the sparrow (48)   The initial "endosteal reaction" may follow estrogen injection alone and consists of the deposition of a few lamellae of endosteal bone   Apparently androgen must also be present to bring about the accelerated endosteal proliferation with rapid formation of medullary bone which occurs in the normal osseous cycle   However, the administration of estrogen and androgen in any combination yet tried has not proved as effective in bringing about hyperossification as is the pigeon's own ovary   Hyperossification takes place at a much slower rate when experimentally produced than when occurring during the sexual cycle   Medullary bone formation may occur with an amount of estrogen which is relatively ineffective in raising the serum calcium level, especially when androgen is administered simultaneously, and androgen does not augment the calcium values found with a low dose of estrogen   Therefore, hyperossification and serum calcium level are not necessarily physiologically interdependent   The intramuscular injection of parathyroid extracts into birds in which medullary bone formation had been induced results in some resorption of bone accompanied by osteoclastic activity   Resorption does not, however, reach the proportions observed in the laying pigeon   It seems probable that the mechanism responsible for bone formation in the normal female pigeon has not been completely elucidated (27)

Chickens   Sexual dimorphism, while not evident in pigeons, is marked in fowl   This dimorphism is quite definite in the skeleton   Hutt (49) showed that a statistically significant sex dimorphism occurs in the mean absolute length of all bones of the appendicular skeleton of the chicken, the longer bones having a 13 4 per cent greater length in males than in females   The increase in length is not constant in all long bones but varies from 6.8 per cent to 16 5 per cent   Some evidence has been advanced that capons have slightly larger bones than do normal males (49), but very carefully controlled experiments indicate that castration of male chicks at hatching does not produce any changes in absolute or relative length of bones of the extremities in either normal or creeper fowl (50)   That large doses of estrogen dwarf the bones of fowl is evident from the findings of Zondek (51) to be discussed later   Asmundson et al (52) have also reported that excessive production of endogenous estrogen in young pullets following administration of pregnant mare serum dwarfs the tibias and tarsals   However, studies on gynadromorphism in the fowl (53 54) indicate that the size differences in the skeletons of male and female birds is genetically, rather than hormonally controlled, since in the gynandromorph the skeleton may be of the male size on

one side and of the female size on the other, yet the hormone action should be the same on both sides   Other skeletal features, such as the width between the pubic bones, are distinctly different in the two sexes and are presumably affected by estrogen (52, 55), although accurate measurements seem to be lacking   Even the most accurate measurements failed to show a correlation between skeletal size and egg laying capacity within the limits of any one breed of chickens (56)

In connection with the stunting of growth which follows injections of large doses of estrogen (1,600,000 m u , or approximately 533 3 mgm of dimenformon in 16 weeks) Zondek (51, 57) observed that in x-ray plates of dwarf cocks produced by this treatment the opacity of the medullary cavity was the same as that of the bone surrounding it   With injections of 50,000 m u (approximately 16 66 mgm) of dimenformon, the first indication of the induction of bone changes could be observed after 4 weeks, if treatment was started at the age of 6 weeks   When the bones were split longitudinally the medullae were found to be filled with spongy bone   The femur showed a reduction of 18 9 per cent in length and of 40 6 per cent in weight, while the tibia was reduced 30 8 per cent in length and 30 0 per cent in weight   However, Landauer et al (35) showed by means of estrogen injections into sexually mature cocks, where no stunting resulted, that the hyperossification was not a result of the stunting but, as in the pigeon, occurred in response to the presence of the estrogen   Three weeks of treatment with a total of 1 19 mgm of estradiol benzoate resulted in the deposition of a thin layer of new bone on the endosteal surface of the femur and tibia   This is an appreciably lower dose of estrogen than used by Zondek, especially when considered in terms of the body weights of the birds, but is much greater than that required for hyperossiffication in the pigeon   Higher doses of estrogen resulted in more extensive hyperossification   The effects of simultaneous administration of estrogen and androgen have not been tested   Therefore, it is not known whether androgen augments the formation of medullary bone in the chicken Histological studies of the bone changes in the chicken have not been published, but it is assumed that they are quite similar to those taking place in the pigeon

Other birds   Bone changes similar to those occurring in pigeons have been observed in ducks (36) and sparrows (37) following administration of estrogen Large doses of estradiol benzoate are required to bring about these effects in the sparrow   In fact, the first attempt to obtain hyperossification in the sparrow was unsuccessful in spite of the fact that osseous changes occur in the normal female sparrow during the sexual cycle just as readily as in the laying pigeon (58) This failure has been attributed to the small doses used   Unfortunately all males tested were juvenals so that any effect an active testis might have was not observed, Evidence that androgen neither enhanced nor inhibited the degree of osseous change resulting from the administration of estrogen has been advanced by Pfeiffer et al (37)   However, these findings should be reinvestigated in the light of the observation that androgen augments the formation of medullary bone in pigeons (27)

The extent of new bone formation differs greatly in the two breeds of ducks which have been tested   Administration of relatively large doses of estradiol

benzoate (2 mgm. daily) to Mallard drakes, a low egg laying breed, resulted in the production of only a limited amount of medullary bone, while the same amount of estrogen administered to Pekin drakes, a relatively high egg laying breed, produced extreme bone changes   However, this material is too limited to give more than an indication that the differences in egg laying capacities influence the response to estrogen (36)

EVIDENCE INDICATING THE EFFECTS OF INTRINSIC SEX HORMONES ON THE DE VELOPMENT OF THE MAMMALIAN SKELETON   *Influence on general skeletal development*   The obvious differences in the sizes of the skeletons of males and females of many mammalian species have been undoubtedly associated with the influences of the gonads for many years.   In addition specific sexual dimorphisms of parts of the skeleton such as the pelves show special sexual characteristics which may be in part genetically and in part hormonally conditioned   Although studies on sexual dimorphism and on the influence of gonadectomy upon the skeleton have infrequently been sufficiently extensive to prove the extent of humoral gonadal control of skeletal morphogenesis, such observations have con tributed significantly to our information   Certain aspects of these investiga tions, especially of those pertaining to man, have not been covered completely in this review, but only sufficiently to indicate the trend and some conclusions of this investigational work.   A few studies have been given more space than they may merit, but reference frequently is made to them in spite of the very limited material upon which conclusions were based

Influence of castration in man   The Scopecs have afforded material for the study of the effects of castration on skeletal growth (59, 60)   Among the Scopecs some individuals castrated before the age of puberty had abnormally longer appendicular skeletons and smaller calvaria than other individuals of similar heights from the same racial groups   Individuals castrated after puberty tended to resemble in stature the intact males of their racial group (61, 62)

A very detailed study was made on 31 eunuchs from North China (63)   Nine eunuchs were castrated when less than 15 years of age   These eunuchs attained heights ranging from 163 to 182 cm and averaging 172 cm   The heights of the individuals castrated when over 15 years of age ranged from 154 to 168 cm and averaged 163 cm at the time they were measured   The average heights to the tops of the symphysis pubes were 92 and 84 cm for the early and late castrate groups respectively   The greater part of the difference in height therefore could be accounted for by differences in length of limbs   The eunuchs were also taller than other individuals from the same region

Time of appearance and union of ossification centers in man   Pryor (64–67) reported that centers of ossification in females appeared at earlier ages than in males.   His studies convinced him that the ossification of the skeleton of the female was always in advance of the male from the earliest appearance of centers of ossification, at first days, then weeks, months and years in advance   Detailed studies were made on the ossification centers of the hand (66, 68)   Other in vestigators did not find the striking sex differences in skeletal maturation de scribed by Pryor (69–72)   Recently complete studies have been made on the

sequences of osseous development of the hand (70, 71, 72)   Although Todd (72) observed a somewhat more rapid skeletal maturation in females he considered it closely related to the progress of sexual maturation, only negligible at the ninth year and not striking until the thirteenth year and from then on until the later teens   The stage of development of the skeleton of boys was associated more closely with evidences of sexual development than with the chronological age (73)

Some evidence exists for the early cessation of growth in cases of pathologically precocious hypergonadism, although growth may be accentuated, at least, for a while (74, 75, 76)   Later, mention will be made of retarded growth (77, 78) and delayed union of epiphyses in hypogonadal patients

Dogs   The skeletal development of an ovariectomized dog was compared with that of an intact littermate (79)   The operation was performed at the age of 3 months and the animals were killed 12 months later   The ovariectomized dog was larger throughout the experiment, the bones of the extremities were longer, some of the epiphyses had not united and the pelvis was smaller than in the control   Significant differences existed between the males and females in the various indices and measurements of the heads of several breeds of dogs (80) The material, however, did not prove that these differences were attributed to the intrinsic gonadal hormones

Sheep   At birth the skeletal development of male and female lambs was comparable (81)   At the end of 5 months the bones of males were larger than those of castrate males, or of females   The bones of the head, neck and pectoral girdles of males were disproportionately large when compared with the females or castrates   Although the bones of the male pelves increased more in weight the relative increase was greater in the female

Cattle   The measurements obtained by Tandler and Keller (82) showed that the skeletons of bulls and cows differed greatly and that the skeletons of castrated males and females were similar and of an intermediate type

Mice   The formation of centers of ossification and the fusion of the epiphyses have been reported in mice but reference was not made to sex differences   The changes of the bones during the late developmental period were apparently similar in the two sexes   Histological studies were made on the distal ends of the femurs and proximal ends of the tibias of mice of seven different strains and from 1 to 30 months old (84)   The epiphysial cartilages of the multiparous females became very thin and the resorptive phase began at an earlier age than in males or virgin females   The rapidity of changes with ageing lagged in males, during the first year of life being 2 to 3 weeks behind the females, but when resorptive processes became accentuated during the second year of life they progressed rapidly and soon the bones showed more resorption than those of the females   The differences in the rate of the ageing process in bones of mice of different inbred strains were apparently much greater than between the two sexes within the same strain

"Breaking strength" and chemical composition   The "strengths" of the bones of male and female inbred C_3H strain and of hybrid mice at progressive ages did not entirely corroborate the above observations (85)   The bones were strongest

in mice from 100 to 300 days old and thereafter decreased progressively   The femurs of the females were stronger than those of the males in almost all groups   Virgin females had stronger bones than the multiparous animals   The strength of the bones was associated directly with the density of the bone as determined by their radio opacity   The per cent of ash and the calcium phosphorus ratio were higher in the femurs of females than of males (86)

Rats—Appearance of ossification centers   The centers of ossification of the limbs of rats appeared at an earlier age in females than in males   The cleared limbs from large series of rats from 62 different litters were stained with alizarine and the number and size of the centers of ossification recorded (87)   Littermate brothers and sisters were compared because the stage of ossification differed con siderably between different litters of presumably the same age   Difficulties in the determination of chronological age or differences in environment were as sumed to be responsible for the variations among different litters   X ray ex amination of intact male rats and male rats castrated at one day of age revealed no effect of castration on the age of appearance or fusion of ossification centers other than the ischial epiphysis and os priapi which were delayed in the castrated animals (88)

Gonadectomy on skeletal growth   Castrate and intact male rats from the same litters attained similar weights (89) although ovariectomized females were 3 4 per cent longer and 23 5 per cent heavier than their unoperated sisters (90)   Male rats castrated when 21 days of age attained adult weights 24 per cent less and had bodies 7 9 per cent shorter than those of the unoperated controls   The tails of the castrated rats were only 3 1 per cent shorter than those of the con trols (91)   Male rats castrated when 21 to 23 days old had shorter bodies and femurs than those of their intact controls when 88 to 105 days of age   The lengths of the tails and humeri of control and castrated groups were similar (92)   Male rats castrated when 22 days of age and killed at 80 days of age were 10 9 mm shorter nose-anal length and 26 grams lighter than their intact controls (93)   Rats castrated at weaning and killed after one year had significantly shorter femurs, tibias, humeri, radii and ulnas than unoperated controls (94)

Spayed rats had longer bodies than hysterectomized controls (95)   Although castrated female rats were 19 per cent heavier than their intact controls their body lengths and the lengths of their femurs were similar at 184 days of age (96)   Rats castrated when 26 days of age had significantly longer femurs, humeri, bodies, and tails when 70 to 90 days of age but not when 184 days old (97, 98)   The rats ovariectomized when young grew more rapidly for a few months but within six months were again no larger than the controls   X ray examination of the skeleton revealed "longer bony structures" in castrated females than in intact controls (99)   Female rats castrated when 40 days of age weighed more when from 80 to 290 days old than virgin females or females on normal or "forced breeding" schedules   The lengths of the tibias of the castrates also were greater during the first few months after castration but were eventually shorter than those of females from which the young were removed at birth   The virgin fe males were smaller and had shorter tibias than any of the other groups (100)

Sex differences on chemical composition   The per cent of calcium in the bodies

of female rats from 15 to 243 days of age was greater than in the males   The difference became definitely significant in animals over 30 days of age and was greatest in sexually mature animals   The absolute amount of calcium was greater in the males because of their larger size (101)   Femurs of female rats from 23 to 150 days old had a higher ash content than did those of littermate males (102)   The per cent of calcium and phosphorus was also higher in the bones of the females (103)   The greatest relative change in the per cent of ash content per unit of age occurred in females at ages ranging from 20 to 30 days and at about 36 to 40 days in the males   These periods of rapid increment of ash were associated with phases of gonadal development (104)

Guinea pigs   Castration on length of bones   Male and female guinea pigs gonadectomized when 15 to 30 days of age and killed when 360 days old had body weights similar to those of the controls   Although both the gonadectomized males and females had shorter bodies, the long bones of the legs were nearly equal to or longer than those of the controls (105)   These experiments in part confirmed earlier work by Bouin and Ancel (106) who found that the femurs and tibias were longer in prepuberally castrated guinea pigs   On the other hand castrated females bearing testicular grafts attained the size of normal males (107)

The tibias, ribs and joints of young gonadectomized male and female guinea pigs were obtained at weekly intervals and were studied histologically and compared with sections from the bones of intact controls (108)   The ratio of hypertrophic to proliferating columnar cartilage cells was 4 or 5 10 or 11 for the male and female controls, 5 16 or 20 for the castrated males and 5 or 6 17 or 18 for the castrated females after five weeks   The epiphyseal cartilage thus increased in thickness in both groups of castrated animals   With degeneration of hypertrophic cartilage cells some calcification of the bars of matrix followed and ossification occurred   The cartilages of the joints also hypertrophied, tending to parallel the changes occurring in the epiphyses   The increased proliferation of cartilage occurring during the first months after ovariectomy eventually ceased and sclerosis and ossification progressed although both at a slower rate than in intact females (109)   Gigantism did not occur, although maturation of the osseous system was delayed

Influence on the dimorphism of the pelves   The shapes of the pelves in man differ in different individuals—especially the shapes of the pelvic outlet   The pelves of children of under 11 years of age were almost all of the anthropoid or dolicopellic type as determined by the roentgenological method of Thoms (110) The pelves of the males and females began to assume their specific sexual characteristics in children approaching fifteen years of age   The observation on 59 female and 16 male children from 5 to 15 years of age thus "suggested that both males and females start out life with pelves which are identical in type and that the major differences observed in adult male and female pelves do not appear until after puberty and are therefore due to the influence of sex hormones" (111) Similar observations had been made by Odiditsch (112), who also noted that chronic disease modified the pubertal metamorphosis of the pelvis

The types of pelves of both males and females ranged widely from dolicopellic

to platypellic but the pelves of males showed 1, more prominent and heavier ischial spines, 2, narrower pubic angles, and 3, narrower greater sciatic notches than those of females. The pelves of the "more masculine" or "more feminine" individuals did not necessarily show the most accentuated characteristics of the respective sexes. In fact two hypogonadal males had typically masculine types of pelves. The morphological characteristics of the pelves were not necessarily a reliable indication of the degree of maleness or femaleness of the individuals possessing them (113, 114).

During pregnancy the pelves enlarged slightly by a loosening of the sacroiliac ligaments and the pubic symphyses. A joint like space was frequently encountered in the symphyses of females (115, 116, 117). The pelvic loosening may be of sufficient magnitude to impair locomotion because of lack of pelvic rigidity. Pelvic mobility, when once it has appeared, may recur with each subsequent pregnancy. Repair may occur following delivery (118, 119).

Sheep. The pelves of newborn lambs showed no sexual dimorphism. In the adult male the pelvis was larger than in the female, composed of heavier bones but longer and narrower than in the female. The pelves of castrated males and females were smaller than those of the females but were similar in shape. True pelvic sexual dimorphism existed and was determined, apparently, by the sex hormones. The castrate type, however, more closely resembled the pelvis of the female (120).

Guinea pig. The bony pelvis has been associated more frequently with reproductive function than have other parts of the skeleton. The guinea pig's pelvis apparently attracted the attention of investigators because of the large size of the young at birth. The separation of the pubic bones of the guinea pig before parturition has been described repeatedly (121, 122, 123).

Virgin guinea pigs had distinct pubic symphyses (124). A chondrofibrous junction of the pubes appears in young virgin female guinea pigs, the amount of fibrous tissue increasing at the expense of the cartilage forming a syndesmosis (125). Some cyclic separation of the pubes may recur with the repeated estrous cycles (126). The fibro-cartilaginous interpubic unions of male guinea pigs were replaced by bone in old animals so that the symphyses were partially destroyed.

During pregnancy the amount of fibrous interpubic tissue increased greatly, especially after the first five weeks (125). Subsequently some bone resorption of the symphysial face of the pubes occurred. The marrow spaces were exposed and the fibrous tissue of the symphysis showed increased vascularization. The proliferation of connective tissue cells was especially rapid during the last two weeks of pregnancy and the pubes became widely separated just before parturition. The interpubic ligaments were shortened directly after delivery but were never completely lost.

Pocket gopher. Two types of pelves have been described in each of several species of Rodentia and Insectivora but the author did not associate them with sex (127). The female type of pelvis of pocket gophers was associated with the presence of functional ovaries—the pelvic skeleton was a secondary sexual character (128). The dimorphism first became apparent at puberty and pro-

gressed with the complete removal of the medial part of the pubes   Orchid-ectomy did not alter the development of the usual male type of pelvis   Ovari-ectomy usually prevented the formation of the female type of pelvis

Mouse   The pubes of male mice were united firmly by fibrous and cartila-ginous tissue throughout life and the amount of interpubic cartilage decreased with advancing age (129)   The pubes were less firmly united in adult virgin females, but the cartilaginous symphysial faces were closely approximated by ventral and dorsal ligaments which enclosed a joint-like space   During preg-nancy the medial parts of the pubes were absorbed and interpubic ligaments formed   After one pregnancy the ligaments were about 2 to 5 mm in length and became longer in mice having several litters   Following delivery the pelvic architecture never returned to the condition of that of the virgin female   The greater part of the interpubic ligaments formed during the latter 8 days in intact pregnant mice, in pregnant animals from which the fetuses had been removed but the placentas retained (130) and in hypophysectomized pregnant mice (131, 132)

INFLUENCE OF INJECTED SEX HORMONES UPON SKELETAL DEVELOPMENT   *Rats —effect of estrogens*   Several investigators have noted that the injection of estrogens inhibited or reduced the rate of increases in body weight   Fewer ob-servations have been made on osseous or skeletal growth but there is general agreement that large doses of estrogens inhibit or stunt growth when injected over prolonged periods

The skulls and long bones of estrogen-treated male and female rats were smaller (133)   Twice weekly 500 I U of estradiol benzoate were injected into rats 4 to 6 weeks old and the growth of the treated rats was compared with the controls After 3 to 4 weeks the growth increments of the treated rats decreased and ceased after 3 to 4 months   Body length was also greatly reduced (51)   Later it was shown that these "eunuchoid dwarf" rats had skeletons more opaque to x-rays than the controls   The medullary areas were invaded by bone   The lengths of the bones were reduced (57, 134)   Zondek assumed that estrogens inhibited not only the gonadotropic function of the pituitary but also the growth-stimulating function (134)

Theelin when injected into young rats at the rate of 20 μg per day had no effect upon body length during experiments extending over 5 to 10 days, although body weight was reduced and the tails of the estrogen-treated rats were shorter than those of the controls (135)   Rats receiving 20 μg of estrone daily from the 21st to the 99th day weighed less, were shorter, and had shorter tails and femurs than the controls (136)   The simultaneous injection of testosterone propionate (1 mgm per day) prevented the decreased rate of gain in weight and length ob-served in estrogen-treated rats (137)

The daily injection of 12 5 μg of estradiol to male and female rats from 1 to 7 or 9 days of age "causes a significant advance in skeletal age of females but not of males "   Treated females had 32 per cent more centers of ossification in their appendicular skeletons than did the males (138)

The oral administration of 50 to 250 μg of stilbestrol daily greatly reduced the rate of growth of young rats (139)

The daily injection of 0 2 to 1 mgm of estrone inhibited the longitudinal growth

of the skeleton of young rats and decreased the thickness of the epiphysial discs
The daily injections of 200 µg of stilbestrol were more effective in stopping
osseous growth. The injection of hypophysial growth-stimulating hormone
prevented the inhibiting action of estrone on the proliferation of cartilage and
bone (140, 141)   Since the epiphysial changes of the hypophysectomized and
estrogen treated rats were not similar it was assumed that the estrogens did not
act by opposing the production of growth hormone but acted directly upon the
growth zone of the epiphysial cartilage (141)   Growth hormone did not com
pletely prevent the inhibition of skeletal growth in animals given large amounts
of stilbestrol (142)

Histological changes   Several factors have been considered in microscopic
studies on growing bones, namely, the proliferation of cartilage at the epiphysial
junction, the sclerosis of cartilaginous matrix and the proliferation of the osteo-
genic tissues at different sites within or about the bone

Young rats given large doses of estrogens for periods of 17 to 26 days had
slightly thinner epiphysial cartilages and an increased number of osseous spicules
in the proximal metaphyses of their tibias   The amounts of osseous tissue were
greater in the females than in the males   Untreated control rats "pair fed" at the
levels consumed by the estrogen treated rats, had thinner epiphysial cartilages
than the ad libitum fed controls but the cartilages were not as thin as in the
estrogen treated rats   The tibias of adult estrogen treated rats showed similar
but less extensive changes (143)

The bones of rats which had received weekly injections of 33 µg to 83 µg of
estradiol benzoate from weaning age up to 17 months were studied by the Silber
bergs (144)   After treatment for 4 months the epiphysial cartilages were thin
and partially sclerosed   The cartilages were sometimes absent in rats treated for
9 or 10 months   The latter condition was observed in some untreated rats 17
months old   The sub-epiphysial marrow-containing areas of the tibias of rats
treated for 4 months were relatively fibrous, contained numerous osteoblasts
surrounding an increased number of newly formed osseous trabeculae, some of
which surrounded sclerosed spicules of cartilage   The apposition of bone had
been excessive during this period   By the 9th month resorption of the osseous
spicules had occurred so that the metaphyses of the bones resembled those of the
controls

A well illustrated report of the osseous changes in rats given 10 mgm pellets
of estradiol dipropionate weekly for periods up to 6 weeks has reaffirmed and
extended a part of the above observations (145)   After one week the epiphysial
cartilages of young estrogen treated rats were thin and the number of osseous
trabeculae reduced, "the cartilage histology resembles that of an animal that had
been almost starved," a probability since appetite was reduced   After 3 weeks
the cartilage had partially recovered but was again reduced after 6 weeks   Dur
ing the first 3 weeks many small spicules formed and later fewer coarse trabeculae
of bone occupied the greater part of the proximal medullae of the tibias   The
increased rate of ossification did not occur in estrogen treated hypophysec
tomized rats (146)

The injection of estrogens was considered to induce localized osteofibrotic

lesions in young rats (147)   This fibrosis occurred chiefly subperiostially near the metaphysis and was assumed to be responsible for the absence of "clubbing" of the bones or for the maintenance of their normal shape during longitudinal growth (148)

On composition   The calcium-phosphorus ratio of the bones of estrogen-treated rats was higher (2.39) than that of controls (2 10)   This change in composition was attributed to a decrease of phosphate and an increase of carbonate in the bone salts since the total calcium was not increased (149)

Body weight was slightly lower but the weights and ash contents of the bones of very young rats which had received 50 to 250 μg estradiol benzoate during 7 to 50 days were similar to those of the controls (150)

The femurs of young or young adult rats which had received estrogens for 44 days or more contained a significantly higher per cent of ash than those of their controls when they were fed an adequate diet (143)   The femurs of estrogen-treated old rats showed no increase in ash during experiments extending over 37 days   In none of the experiments was the total ash increased significantly in the estrogen-treated animals because of the decreased growth of these rats   Estrogen-treated rats ate less than their ad libitum fed controls   "Pair fed" controls gained more weight than the injected rats but their femurs contained less ash and also a lower per cent of ash   The decreased food intake could account for neither all of the decreased gain in body weight nor the increased per cent of bone ash Bone phosphatase increased significantly in rats treated for 17 days

Repair of fracture   Callus formation occurred somewhat sooner about fractures of femurs of castrated rats given estrogen than in the untreated controls (151)   Although the effects upon the fractures were slight the authors "gained the impression that injections of estrogenic substances stimulated the production of endosteal osteoid tissue," although the fractures in the different treated and control rats were at different and overlapping stages of repair (152)

Androgens—Size of bones   Androgens, especially testosterone and its esters, have been reported to either stimulate or inhibit body growth or growth of the skeleton   Factors such as the dose of hormone given, the duration of treatment, and whether or not the animals were castrated may influence the results   These factors will not account for all of the opposing results that have been reported Daily doses of testosterone of 200 μg did not alter growth in young rats treated for 1 or 2 months (153)   Doses of 1400 μg of testosterone propionate reduced the weight-increment in another group of young rats while smaller doses (167 μg) stimulated growth (154)   Young rats given 35 mgm of testosterone propionate during 40 days gained much less weight and had shorter bodies, tails, femurs and tibias than oil-injected or untreated controls (155)   The epiphysial cartilages were thin and atrophic   Testosterone propionate (1 mgm per day) increased the rate of gain in weight and length of young rats and prevented the growth-inhibiting effect of estrone (137)

Male rats given 1 mgm of testosterone propionate daily for 80 days weighed less and were shorter than untreated controls   Differences in weight and length were not apparent at the 26th day of injections but the controls were 6 5 mm

longer at the 80th day (156)   Castrated rats were on the average 11 mm shorter than intact untreated controls and castrated rats given 1 mgm of testosterone propionate daily were 13 5 mm shorter   Again the differences were not apparent until at the 26th day (157)   Castrated and intact rats given small doses of testosterone propionate (50 µg) 6 times weekly increased in weight more rapidly than the controls (158)   Although no measurements were taken the weight changes of young rats, some injected from the first day of life and given from 1 mgm of testosterone propionate 3 times weekly to 5 mgm daily, did not differ greatly from those of the oil treated controls (159)   Some of the experiments were continued for 25 weeks

The os priapi and ischial epiphyses appeared much earlier in male rats given 0.25 mgm of testosterone propionate daily or every other day but after 87 days there was no significant difference between the treated and control group (88)   A systematic x ray examination for the time of appearance and fusion of many other centers of ossification showed no difference between controls and treated rats. Doses of this size or larger (1 or 2 mgm daily) did not alter body weight or skeletal maturation even when started at birth and continued from 16 to 135 days

Upon os priapi   Many studies have been undertaken recently on the influence of sex hormones on intrauterine or newborn rodents   None have shown effects of hormones upon the skeletons of the young   Estrogens will apparently partially inhibit the development of the os priapi in fetal males (160)   Castration immediately after birth did not prevent the development of the os priapi in the male but the os failed to develop in the masculinized clitoris of rats given small doses of androgen after birth (161)   Large doses of androgen injected into the pregnant mother induced well developed os priapi in the intersex female young born under such conditions (162)

Mouse. Effect of steroid hormones on gross changes   Although the pubic bones were partially resorbed in estrogen treated mice the density of the other bones of the skeleton was increased (129)   The medullae of the long bones of mice which had received 3.3 to 33 3 µg of estradiol benzoate or 0 1 mgm of equilin benzoate weekly for prolonged periods contained either excessive numbers of fine or coarse osseous trabeculae or, after more prolonged treatment, compact bone (163)   The progression of changes depended upon the amount of hormone used and duration of treatment   Similar gross skeletal changes were induced by injections of estrone, equilin, triphenylethylene, and stilbestrol (165)   The bones of mice of some strains responded more rapidly than others

The simultaneous administration of 0 625 to 2.5 mgm. of testosterone propionate completely prevented the excessive accumulation of medullary osseous tissue in the long bones of estrogen treated animals (164, 166)

"Breaking strengths' and composition   The bones of estrogen treated mice had a higher ash content (69 per cent) than those of control males (59 per cent) or females (64 per cent) and a higher calcium phosphorus ratio (86)   The ash content of the femurs of mice receiving testosterone propionate or estrogen plus testosterone propionate was 60 per cent hence approached that of the normal

males   The new endosteal (medullary) bone apparently had a slightly higher
calcium-phosphorus ratio than the bone of the diaphyseal shaft

The structure of the bones of estrogen-treated mice differed from those of
the controls as determined by x-ray diffractograms   The bone in the former
medullary region was entirely unoriented and the diffractograms differed from
those obtained from cortical bone or bones of untreated mice (167)

The femurs of estrogen-treated hybrid mice had "breaking strengths" of 1599
to 3542 grams (average 2499 grams), whereas femurs of controls of the same
age groups had "breaking strengths" of 1244 to 2101 grams (average, 1655
grams)   The average "strength" of the femurs was thus 844 grams greater in
the mice which had received weekly subcutaneous injections of 16 6 to 50 μg
of estradiol benzoate (168)   Similar observations have been made on a larger
series of mice (85)   The "breaking strengths" of the femurs of estrogen-treated
males were greater than those of the estrogen-treated females   The differences
in "breaking strengths" of femurs between the estrogen-treated and control
groups tended to increase with advancing age   The estrogen tended to prevent
the thinning of the diaphyseal walls associated with advancing age   The bones
of the mice given testosterone propionate had very low "breaking strengths "

Histological changes   The bones of estrogen-treated mice became almost
solid, or the medullae were filled with spicules greatly reducing the marrow   The
apposition of bone was entirely endosteal—no evidence of excessive periosteal
growth was observed (163–166)

A roentgenographic and histological study of the femurs of mice of two strains
which had received weekly doses of 150 to 1000 r u of estradiol benzoate showed
sclerosis of the cartilage and "osteoblastic activity" leading to generalized osteo-
sclerosis   The long bones were first and most extensively affected but the verte-
brae and calvaria also showed excessive osteogeneses and delayed resorptive
processes (169)

Detailed and comparative histological studies have been made on the bones
and articular cartilages of young and old estrogen-treated mice from several
inbred strains (170, 171)   The mice received different levels of hormone for
different periods of time   Within 1 to 2 weeks after the injection of estrogens
was started the epiphysial cartilages were reduced in thickness and the number of
layers of hypertrophic and columnar cartilage cells was reduced   The cartilagi-
nous ground substance became hyalinized and sclerosed   The reduction of the
width of the cartilage and extent of hyalinization and sclerosis of cartilage matrix
increased in mice treated for longer periods (3–6 weeks)   With more prolonged
treatment calcified matrix or bone separated the epiphysial cartilage from the
marrow and in some areas the cartilage was completely eroded, exposing the
marrow of the epiphysis and diaphysis

The vascular marrow of the metaphysis became less vascular and increasingly
fibrous (170)   "Epithelioid" cells appeared in the fibrous marrow after treat-
ment for 2 weeks   These cells then increased in number and "became con-
verted" into osteocytes   New bone matrix was formed by these osteocytes
forming first small and then larger spicules and finally a dense bony meshwork
Resorptive processes were largely inhibited during this time   After 2 or 4

months 'osteoclastic resorption" set in and the bone was gradually rei
until, at 9 months, few metaphyseal trabeculae remained   The diaphysea
were thickened during the first 4 to 6 months of treatment and thereafte:
reduced gradually in thickness

The extent of the bone changes was greater in the males than in the fe
The bones of mice of some strains were affected to a much greater exten
those of other strains

When the injections of estrogens (100 to 200 r u weekly of estradiol ben
were started in adult (6 to 11 months old) or in aged mice (12 to 18 mo
less or no increase in the thickness of the metaphyseal spicules or diap
walls occurred   The age changes in the epiphyseal cartilages were accen
and the resorptive phases stimulated precociously rather than inhibited
liferative arthropathic lesions occurred less frequently than in untreated co
Estrogenic treatment accentuated rather than inhibited the usual reso
phases of the ageing process when the injections were started in old anime

The proliferation of cartilage and resorption of bone were also inhibi
young mice given 0.25 to 0.85 mgm of testosterone propionate weekly
weeks or more (172)   The changes were similar to those occurring in est
treated mice but much less striking

Guinea pigs   Guinea pigs which received 50 to 100 I U of estradiol bei
daily for 27 to 38 days had thinner epiphysial cartilages than the contro
layer of hypertrophic cartilage was especially reduced (173)   The inunct
injection of estrogens decreased the rate of gain in weight and longit
growth of the tibias of young guinea pigs (174)

The daily injection of 20 r u or biweekly injection of 250 r u of est
benzoate into male and female guinea pigs weighing 140 to 190 grams inc
the rate of hyalinization of the cartilaginous ground substance of the epip
discs (175)   The amount of the hypertrophic cartilage first decreased an
increased while the proliferative cartilage showed an inverse relationship
fication and sclerosis of the cartilage were increased   Metaplasia of the col
and hypertrophic cartilage cells to osteocytes was described   In the metap
'epitheliod" cells replaced the vascular marrow and osteocytes appeare
formed spicules of bone and abundant osteogenic tissue   Because reso
was reduced an increased amount of bone appeared in the metaphysis
diaphyseal shaft was thickened and became denser during the first few m
but later became thinner and more porous as resorptive processes becan
centuated   Animals of both sexes responded similarly   The doses we:
large enough to prevent growth although the estrogen treated animals gre
than the controls (174, 175)

Estrogen treated guinea pigs, one week old, when given 50 to 100 i
estradiol benzoate weekly showed first a decrease in the thickness c
epiphysial cartilage and then an increase (175)   The layers of both th
liferative and hypertrophic cartilage increased in thickness and the ten
for sclerosis of cartilage and osteoblastic proliferation and ossification
reduced appreciably

Progesterone increased the proliferation of columnar and of hypert

cartilage and inhibited sclerosis and retrogressive, changes in cartilage when injected in the amounts of 0 65 to 1 mgm 6 times weekly (176) Bone formation was relatively reduced "thus counteracting the aging of bones "

Dogs Two young dogs treated with estrogen (Menformon) showed precocious union of the epiphyses (177) Puppies given 1 66 to 3 33 mgm of estradiol benzoate weekly for 5 to 7½ months, weighed less and were shorter than untreated dogs The epiphyses were united precociously The pubic bones were not altered and in the males the growth of the os priapi was inhibited (178)

Man—Estrogens Very few studies have been undertaken in man on the effects of administered estrogens on osseous growth or on bone salt retention The daily injection of 2,000 I U of estrone to young girls for 6 to 18 days resulted in a depression of the calcium retention The amount of hormone injected was adequate to cause mammary swelling and a vaginal discharge (179)

Albright and his associates have associated post-menopausal osteoporosis with a deficient production of estrogenic hormone The post-menopausal osteoporosis most frequently involved the vertebra and the pelves, the long bones were rarely involved (180, 181) The injection of estrogen into such patients resulted in a positive calcium retention and marked subjective improvement Nitrogen retention was also increased

Androgens The occasional occurrence of hypogonadism and retarded growth or of retarded genital and somatic growth probably indicate the desirability of the following investigations Children showing retarded sexual development, sub-normal growth and a sub-normal bone age showed accelerated growth when gonadotropic hormones were injected (182, 183) Although the rate of growth was much in excess of that expected from standard growth charts the bone-age changes were not proportionally accelerated (184, 185, 186) The secondary sexual characters also usually developed indicating gonadal stimulation

The possibility that the accelerated growth resulted from the androgenic activity of the stimulated testis was investigated by direct injection of androgens into similar patients One 21 year old eunuchoid individual gained 4 cm during the 6 months of treatment with testosterone propionate (10–30 mgm daily) (187) The injection of testosterone propionate or implantation of pellets of the hormone lowered the age of epiphysial appearance in 1 young patient but did not greatly accelerate rate of union of epiphyses in adult hypogonadal individuals (188) Spurts of growth were observed in adolescent hypogonadal individuals given testosterone propionate but the rates of epiphysial closure were not sufficiently altered to be detected by x-ray examination (189) Accelerated increase in height has been reported in a eunuchoid patient given testosterone (190) Either testosterone propionate or methyl testosterone increased the rate of growth of hypogonadal individuals beyond that expected Skeletal age, as determined by epiphysial union advanced more rapidly than might be expected from changes in chronological age (191, 192) The frequent occurrence of growth rates in excess of those expected from comparison with charts of normal growth was noted in hypogonadal boys given either gonadotropic hormones or testosterone Bone-age was usually not altered greatly (193)

Patients showing post-menopausal osteoporosis showed increased retention of calcium and phosphorus when androgen was administered in combination with estrogens (182)

Influence of injected hormones on the pelves of guinea pigs   Hisaw (194) apparently first attempted experimentally to induce a separation of the pubes of virgin guinea pigs similar to that occurring at parturition   The pubes of some female guinea pigs became separated by an interpubic ligament when the blood sera of pregnant rabbits, dogs, and other animals were injected   The animals which responded were at or near estrus when the injections were made   Later *the relaxation inducing substance (relaxin) was found in the placentas and corpora lutea of certain animals* (195) and was effective only in guinea pigs which recently had been under the influence of estrogens, either intrinsic or injected

Several other investigators noted that estrogens would induce some ligamentous proliferation at the symphysis as determined by mobility of the pelvis and x ray examination (196–199)   Progestin was not effective by itself or when given with estrogens (196), although a non-estrus producing "extrait mobilisant" could be obtained from corpora lutea   Other investigators have observed an extensive separation of the pubes of young, intact or ovariectomized guinea pigs which received estrogens (estrone or estriol) for prolonged periods   Short periods of treatment with estrogens alone or with progesterone resulted in slight pelvic changes (200, 201)   More recently estrogen and progesterone when administered simultaneously induced extensive pubic separation and the identity of "relaxin" as a specific hormone was questioned (202)

Although the chemical identity of 'relaxin' has not been determined and some investigators have doubted its specificity of function, blood of pregnant laboratory animals and of women apparently contains a substance capable of inducing a rapid pelvic relaxation in estrous guinea pigs   Hisaw first detected the substance in the blood of pregnant rabbits, but not in women   Other investigators found that 3 to 6 cc of blood sera from women during the first half of pregnancy would induce pelvic relaxation in approximately 75 per cent of estrous or adequately treated guinea pigs and that larger amounts were more effective (203, 204) [1]

The experiments on the relaxation of the pelvis of the guinea pig are not in agreement   Few investigators have claimed as rapid and/or extensive a pubic separation as occurs during pregnancy from any treatment with estrogens and progesterone   The presence in blood of pregnant animals, early pregnancy in man, and in aqueous extracts of corpora lutea of a relaxation inducing substance *(relaxin) has been observed repeatedly*   These experiments indicate

[1] Recently Hisaw and his associates have added further evidence for the specificity of "relaxin   Although the pelvic ligaments of castrated guinea pigs would relax when large doses of estrogen and progesterone were given such changes would not occur in hysterectomized castrated guinea pigs   Relaxin on the other hand was effective in hysterectomized estrogen treated animals   Estrogenic or progestational activity could not be detected in animals given large doses of relaxin   References—Hisaw F L et al., Anat. Rec 84 7 abst 1943 A A Abramowitz et al Anat Rec 84 6 abst 1943.

that the active material occurs commonly in pregnant animals and is not one of the known steroid hormones

Mouse   Almost simultaneously two investigators reported that estrogen-treated mice showed a ligamentous separation of the pubes (205, 206)   The interpubic ligaments were readily discerned upon x-ray examination of the carcasses or upon gross examination   The ligaments varied in length up to 7 or 8 mm   Estrone, estriol, estradiol, equilin and equilinen induced similar changes The injection of estrogens thus resulted in a transformation of the pubic symphyses of males or virgin females to the type of interpubic connection found in multiparous females

The interpubic ligaments were formed by a proliferation of the fibrous connective tissues of the ventral and dorsal fibrous ligaments and periosteum   Resorption of the medial parts of the pubic and ischial bones also occurred   When the injections were started in very young animals the pubes were sometimes largely resorbed   The injection of testosterone inhibited the pelvic changes when administered with estrogenic hormone (164)   It likewise largely prevented the pelvic changes occurring during the latter part of pregnancy in primiparous mice (207)

Although quantitative studies on the sacro-iliac ligaments have not been made, the impression was that mobility was increased at this point   The parts of the innominate bones not resorbed showed a slight increased ash content as did the femurs (86)   The shapes of the innominate bones of male and female mice differ from one another, the ischial angle being greater in the female   The injection of estrogens did not alter the male type of ischial angle, even when the injections were started in young animals (129)

INFLUENCE OF SEX AND OF STEROID HORMONES UPON SERUM CALCIUM LEVELS

Rats   Parathyroidectomized or castrated rats showed no change in serum calcium levels subsequent to injections of 600 m u of theelin (209)   On the other hand, the serum calcium levels of intact, hypophysectomized or gonadectomized rats were increased uniformly after the injection of several different estrogenic chemicals (30)   Marked elevations of the levels of serum calcium were observed in estrogen-treated rats in another laboratory and in addition the amount of ingested calcium retained was increased during the first few days of observation and later decreased (210)   The serum calcium levels of young rats after 6 to 12 daily injections of 100 I U of theelin were slightly elevated (211)   The levels of serum calcium were slightly but significantly increased in rats which had received estrogens and in some groups the serum phosphatase was decreased (143)   Other investigators have reported a slight increase in the phosphatase content of femurs of rats receiving estrogens and androgens (212)

The serum calcium levels of estrogen-treated rats usually increased above pretreatment levels   This increase may be slight but statistically significant (143, 211) or of greater magnitude (30, 210)

Rabbits   Adult rabbits injected with crude ovarian extracts presumably containing some active material, showed a slightly lower serum calcium within 24 hours (213)   A rapid and rather marked decrease in serum calcium occurred

within a few hours after the injection of large amounts of estrone into spayed rabbits (214) Other investigators observed no significant changes in serum calcium in rabbits given extracts of ovaries (210, 215) or in rabbits which had received 100 μg of estrone daily for 15 to 31 days (212)

Although high serum calcium levels have been observed in castrated rabbits (214) this observation has not been observed consistently (210)

Dogs Dogs showed a slow but marked rise in serum calcium after ovariec tomy (216) Slight seasonal variations in the levels of serum calcium were noted in female dogs The higher levels were associated with the seasons during which estrus occurred (217) Two dogs which had received 300 r u of estrone daily for 7 days showed an elevation of 2 mgm per cent of serum calcium (30) The total and diffusible serum calcium levels of other estrogen treated dogs in creased about 2 mgm per cent and again returned to normal levels after the cessation of treatment (218)

During proestrum or estrus tetany developed in thyroparathyroidectomized dogs sometimes without appreciable changes in the amount of serum calcium (219, 220, 221)

Parathyroidectomized dogs given estrone or theelol showed a decrease in serum calcium and mild or severe tetany Intact dogs showed no serum calcium changes after estrogenic treatment (222, 223) Tetany occurred during the onset of spontaneous estrus in parathyroidectomized dogs maintained on minimal amounts of vitamin D and calcium The blood calcium levels of rachitic dogs decreased when theelin was injected (224)

Cattle The serum calcium levels of male and female cattle at different ages and following castration were determined by Frei and Emmerson (225) Slight differences between the different groups were noted During estrus the serum calcium level rose in some animals The injection of small amounts of estrogen was followed by a decrease in serum calcium Following the injection of large amounts (20 0 to 120 0 mgm) of estradiol benzoate the serum calcium levels of lactating cows were lowered and the serum phosphatase and inorganic phosphorus tended to rise (226)

It is difficult to generalize on the action of estrogens on levels of serum calcium in mammals The observations even within individual species are not con sistent, and are more variable when different species are compared Most of the studies have been deficient in that certain factors such as food intake and dietary regime were not followed However since the alterations in serum calcium in the mammalian species are so slight it is probable that, if anything, they merely reflect changes occurring in the osseous or other tissues concerned with the utilization, storage, absorption or excretion of calcium There is as far as the reviewers know, no evidence that estrogens influence calcium metab olism in the same manner as do the parathyroids or the "vitamin D ' compounds

RÉSUMÉ

Oviparous animals which have oviducts that secrete or deposit calcified shells around their ova have an obvious need for some mechanism to co-ordinate certain

aspects of calcium metabolism with reproductive function   Since the bones act as storehouses for the calcium reserves of the body, these tissues might also be associated with reproductive functions   The drain upon the maternal calcium stores in mammals imposed by embryonic skeletal development or by lactation might indicate a need for some mechanism of interrelationship between reproductive function and calcium metabolism in mammals

The injection of estrogenic hormones into birds increased the levels of serum calcium and accelerated the formation of endosteal bone   However, the rapid proliferation and ossification of osteogenic tissue could occur in estrogen-treated birds in the absence of marked changes in the levels of serum calcium especially when small amounts of androgen were also injected   The steroid hormones therefore may influence both bone proliferation and the mechanism regulating calcium levels, but these two phenomena are not necessarily related   In some laboratory rodents the sclerosis of cartilaginous matrix, proliferation and ossification of medullary osteogenic tissues were augmented in estrogen-treated animals, but the levels of serum calcium were unchanged or only slightly altered   Androgens prevented the excessive osseous growth when administered to mammals given estrogens

At this time the reviewers believe that the augmented medullary proliferation of bone may be most satisfactorily explained by assuming that estrogens stimulate osteoblasts or the differentiation of esteoblasts from undifferentiated elements of the marrow   The apparent differences in the response of the osteogenic tissues of birds and mammals to androgens when injected simultaneously with estrogens may be due to some fundamental differences in the changes induced in the cells of the marrow

The gonads or the steroid sex hormones regulate to some extent the morphogenesis of the skeleton and may control in part the extent of skeletal growth   Large amounts, especially of estrogens, inhibit the growth of cartilage and hence longitudinal osseous growth   The function of the pituitary gland is probably also altered in such animals and may be directly responsible for the ensuing "dwarfism "   Small amounts of hormones, especially of androgen, may augment the rate of longitudinal growth of the skeleton

The steroid hormones may find a practical application in the prevention or alleviation of symptoms of senile osteoporosis, in the acceleration of the healing of fractures or in the augmentation of the rate of somatic growth in certain hypogonadal individuals

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CHRONIC MOUNTAIN SICKNESS

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The ecological milieu of the high plateaus of the Andes (2 to 5 km altitude) must be emphasized as a fundamental factor when considering the biology of races acclimatized there since prehistoric times (Bingham) "Habitable" (Monge, 1928) places, in which life is naturally possible, must be differentiated from the higher "inhabited" ones, as for example the mines, in which conditions for living must be provided. Such a distinction has not yet been made. When studying acclimatization, much confusion would be avoided if the aim of the investigation were the physiological fitness of the autochthonous man in his ecological surroundings. The sufferances and tolerance of the living body to reach the highlands or to live at an extreme altitude should be considered as correlated subjects but ones of different significance. In no case should one use the words "acclimatized" or "acclimatization" when referring to an organism suffering from climatic anoxic injury during the adaptative stage (mountain sickness)

Congenital acclimatization to the Andean high plateaus (2 to 5 km altitude), where live some twelve million individuals, has produced a climatophysiological variety of human being whose biological characteristics are somewhat different from those of the sea level man. Infertility of men and animals brought from the lowlands, as sometimes occurs (Monge), means the elimination of the unfit through a process of natural selection

When a man goes to the highlands, he has first to adapt himself to a condition of permanent anoxia, calling forth his emergency adjustments and then building up a new set of biological devices to reach the balanced state of the altitude man homeostasis. Consequently, adaptation, itself, is a malady: mountain sickness, either inapparent, acute, subacute or chronic. When adaptation is over, after months or years, no one knows how long, acquired acclimatization supervenes. Then mountain sickness has been cured (Monge, 1928). Acclimatization can also be lost as a shift from the Andean man homeostasis, the patients developing subacute or chronic mountain sickness. If they are brought to a lower land or to sea level, chronic mountain sickness is also cured. In those facts one can find the strict foundation of a climatopathological variety of human disease (Monge, 1, a-h). Animals are subject to the same pathology and possibly plants, although no investigation has been conducted with them. Some men find it impossible to live even at an altitude of 1 5 km. usually such men change their habitat and the observation ends. One could speculate about the condition of a man losing his acclimatization at sea level oxygen pressure

To understand the physiopathological deviations during the adaptative period toward acclimatization, namely, mountain sickness, one must briefly consider the established characteristics of the autochthonous man. The emphasis will be laid upon the facts concerned with chronic mountain sickness which appears as

the basic cause of the problem. Since for some persons, apparently acclimatized, infertility may occur the physiology of reproduction in high altitude will be treated

Chronic mountain sickness as has already been said, is a disease due to unfitness of the individual to become acclimatized at a high altitude or to a loss of acclimatization. Barcroft's work really dealt with sub-acute mountain sickness (2) I first used (1925) the name of Erythremia of high altitude when I had found an erythremic clinical syndrome similar to Vaquez' Erythremia or Osler's Polycythemia vera. This denotes a clinical entity and not a hematological pattern, as some have erroneously interpreted it. In using 'erythremic symptoms,' I refer to a clinical manifestation and not to a high count of red cells. In this review no attempt will be made to study acute mountain sickness[1]. Since I referred to the chronic condition as chronic anoxia I called the disease chronic mountain sickness (1928) (1c d, e). Later, this disease was studied by Monge, Encinas and associates, and Rondon (3) Talbot and Dill (4) found a case of it in Chile. Hurtado described some forms of the disease as related to fibrosis of the lungs (1930 5a), and some to an erythremic syndrome (1942, 5b c, d) Monge (1f) and Arrellano (6) have described neurological forms. Psychiatric forms have been described by Monge (1g)

In natives it may occur at any age and has an impressive familial character (Monge, Rondon) In individuals from the lowlands the onset may come from two to three years after acquired acclimatization and generally appears in the fourth and fifth decades of life. Both sexes are equally predisposed. Certainly it is much more frequent in men of European descent. It may start insidiously or may come suddenly after violent physical exercise or after some respiratory ailment. Repeated attacks of acute mountain sickness predispose as do probably pneumoconiosis, pneumonia and syphilis of the lungs.

I shall first describe the sub-acute forms which when cured lead to altitude fitness. Then I shall consider the chronic stages of the disease.

SUB-ACUTE MOUNTAIN SICKNESS AND THE ANDEAN MAN. The anoxic injury acting on the organism causes an illness which even in its mildest expression, leads to a lessened physical and mental capacity. To avoid confusion I characterized (1925-1928) this as acute, sub-acute and chronic Soroche. This word in the Quechua language means mountain sickness

Subacute mountain sickness is not always preceded by a preliminary attack of acute mountain sickness. In the most benign forms the patient goes on with his daily work, but he realizes that he is unable to maintain the life he was accustomed to at sea level. Even minor ailments provoke a run down condition never encountered at sea level. Diseases are poorly resisted and convalescence is pro-

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[1] This is fully treated in Haldane J S and J G Priestley. Respiration. New Haven, Yale University Press 1935. Barcroft J. The respiratory function of the blood. Cambridge University Press 1935. Loewy A. Physiologie des Höhenklimas. Springer Berlin 1932. The pathology of high altitude climate. Oxford Medical Publications 1937. Dill D B. Life heat and altitude. Cambridge. Harvard University Press 1938. Van Liere E. J. Anoxia its effects on the body. The University of Chicago Press 1942.

lónged   Toxic symptoms accompany pregnancy, delivery is dangerous, mis-
carriages are frequent   Infertility is common   In the more marked cases the
symptoms appear insidiously and one or another predominates as, presumably,
some particular organ suffers most damage from the prolonged oxygen want
General fatigue, cyanosis after exercise, diminished capacity for physical and
mental work, fullness of the head, cephalalgia, dizziness, congestion of the mucous
membranes, respiratory distress, poor appetite, slow digestion, constipation or
diarrhea, loss of weight, insomnia, matutinal tiredness, relieved in the course of
the day as in neurasthenia, are the most frequent complaints   Sometimes the
patient may temporarily overcome his condition   After several weeks, months
or years, the length of time is not yet established, there comes a marked improve-
ment, sometimes abruptly   Very frequently the subject is unaware of his alle-
viation and finds himself fitted to carry on a normal life   He has become accli-
matized   On the other hand, if the illness is more pronounced, fatigue comes
sooner, cyanosis is permanent and nausea as well as vomiting may appear
Hoarseness and dyspnea are the rule   Torpor, cephalalgia, dizziness, vertigo,
nervousness, insomnia, varied algesias and paresthesias appear   The patient,
unfit for acclimatization, is suffering from chronic mountain sickness

   Circulation   In normal individuals, the second heart sound is duplicated very
frequently   Rotta, by means of phonocardiography, has established the proper
significance of this third sound as auricular   The heart rate tends to be markedly
slowed (Monge, Cervelli)   The pulse rate is 40–50 in 13 per cent, 52–60 in 41
per cent, 62–71 in 32 per cent, and 72–84 in 14 per cent (1927)   This has been
confirmed by Talbot and Dill (4), Aste (7), Cervelli (8), Torres (9), Arnaez (10)
and Capdehourat and associates (11) (1937)   The linear relation between work
(700 *kgmts*) and heart rate is only found in 30 per cent of the cases, with an even
slower frequency than at sea level, 70 per cent show a kind of athletic response
After double the amount of work (1,400 *kgmts*) the heart rate is slower than
after a basic work of 700 *kgmts*   A paradoxic bradycardia occurs in 50 per cent
of all cases after moderate work, then the pulse accelerates to reach the normal
level (Monge, Encinas)   Rondon found it to occur at an altitude of 5 8 km
Bradycardia sometimes comes suddenly   In 80 per cent of the cases tachycardia
starts suddenly without any clinical disturbance   At any time the rate may
return to bradycardia   Efficiency tests during the tachycardia do not interfere
with the initial acceleration (Monge and associates 1h, 12)   The electrocardio-
gram in two hundred observations shows, in general, normal values (Monge,
Saenz, 13)   There is a marked sinus arrhythmia, the mean difference between
two systolic intervals may rise to 0 44   After moderate exercise (700 *kgmts*),
I have found exaggerated sinus arrhythmia, shift of the pacemaker, deformity of
P, inverted P wave, shortness of PR, premature auricular and ventricular beats,
voltage changes of T, reversal of T, and ST displacements   Some of these changes
are similar to those found by Greene and Gilbert in acute experimental anoxia
(1925)   The subjects showed no signs of fatigue during the effort tests, not-
withstanding the slow heart rates   Sometimes I have seen a regularly recurring
arrythmia, prolonged (auriculoventricular) conduction, occasional nodal or high

bundle premature beats   In one case the intraventricular conduction was altered   First degree block has been found   There is often right axis deviation   In one hundred cases Saenz found 40 per cent right axis deviation, Rotta found 37 per cent   The venous pressure is increased (Rotta, Capdehourat)   There is a universal dilatation of blood vessels, rather pronounced in sub acute mountain sickness   The arterial pressure in men is reported by Torres, on the basis of 100 observations, to be slightly lower than at sea level.   Rotta's work (14) on circulation can be summarized as follows   a   Systolic output is approximately equal to that at sea level,   b   The cardiac index, of greater importance (Cantoni), reaches 2 3 liters per minute, 12 per cent higher than the value at sea level

The work of the heart in reference to surface area is increased   The volume (in men) and the weight (in dogs) of the heart is greater than at sea level   In sub-acute mountain sickness, tachycardia is the rule but the appearance of unexpected basal bradycardic rhythms (Monge, Cervelli) and paradoxic bradycardias after moderate exercise are common (Monge, Encinas)   In such cases dyspnea is accentuated   At rest, the appearance of a third sound is easily noted   The second pulmonary sound is accentuated   Capillaries are dilated and lose their tortuosity   The arterial pressure is slightly increased with a lower diastolic value (Torres, 9)   Pulsation of the head, as in aortic insufficiency, has been observed (Monge, 1928)   The capacity for work is enormously reduced   Heart efficiency tests show retardation.   Athletes, at high altitude, never maintain their sea level records for very long, as they are in the same situation as are untrained men at sea level   It must be emphasized that outside of the ecological milieu even at the same altitude, the physiopathological deviations are more accentuated   therefore, in the future, it will be necessary to indicate clearly the places where research in chronic mountain sickness is being carried out

Respiration   In the Andeans the chest is enlarged (Charvin, Barcroft (2), Hurtado (5c)) and vital capacity is 10 per cent greater than in Europeans (Barcroft)   Linch (15) found in fifty Andeans that the respiratory rate is slightly increased above the sea level value   After moderate exercise, in 14 per cent, bradypneic rates started either following a short polypneic stage or at the beginning   Sometimes polypneic rates appeared without returning to normal values   In no case did the individual show a particular fatigue   The bradypneic stage is interpreted by Linch as an effort to increase the time of oxygenation.   Arnaez (10) has pointed out, in addition to the well known periodic breath, apneic phases (25 per cent) at rest deep sudden respiration (20 per cent), and staircase breathing as if the first breath were frustrated   This last finding is rather improved after moderate exercise   He suggests that the vagal hypertonia plays a more important rôle than at sea level

These findings are the rule in sub-acute mountain sickness and of course, are much more pronounced   Clinical observation shows that periodic deep breathing accompanying periodic work, is the mechanism best suited to altitudes   Andeans can carry a load of a hundred pounds—they run, stop, take a few deep breaths, and run again   In subacute anoxia, it is possible to ameliorate the symptoms reproducing the periodic hyperventilation of the Andeans   Respira

tory training is important   The respiratory adaptation to anoxemia has been studied by Forbes, Barcroft, Monge, Hurtado and Leon   Vital capacity is considerably increased (Hurtado and Leon)   The most important work has been carried out by Hurtado and Rotta (16), from whom I quote a summarized report

	ABSOLUTE VALUES		RELATIVE VALUES (TOTAL CAPACITY 100%)	
	3 7 km altitude	Sea level	3 7 km	Sea level
	liters	*liters*		
Resp air	2 11	1 50	33 2	26 1
Mean capacity	3 58	2 89	56 4	50 3
Vital capacity	4 18	4 30	66 8	73 8
Total capacity	6 28	5 85		

According to them, the higher total capacity and the absolute and relative increase of residual air suggest a functional compensatory emphysema, as Hurtado pointed out (1928)   This condition disappears at sea level, so it is not a truly acquired but an easily reversible characteristic   During subacute mountain sickness, the vital capacity is decreased, to rise progressively with amelioration   The respiratory form and rate acquire the modalities of Andeans after exercise, with the appearance of signs of dyspnea and asphyxia   Hyperventilation may lead to tetanus   One can observe the anarchy of the respiratory control shallow respiration followed by deep breathing, periodic and Cheyne-Stokes breathing, sudden deep inspiration, apneic phases with sensations of impending asphyxia rather pronounced at night   The last may awake the patient and disturbs sleep (Arnaez, Linch)

Mori-Chavez (17a) has pointed out, in acclimatized guinea pigs, a hyperplasia of the capillary bed and diminished arteriolar structure which permit an increase of the O_2 diffusion surface, facts which must be related to the common observation (Orbigny) of a greater lung size   In the Andean, the chest x-ray has a characteristic appearance   The lung transparency is greater than at sea level, the hilus shadow is noticeably enlarged, and the pulmonary trunks are considerably emphasized, according to Garcia Rossell (18), and Saye and Monge   In sub-acute mountain sickness, this condition is more accentuated   In some cases pulsation of the hilus shadows appears on fluoroscopy   In chronic cases, the vessels' dilatation gives the aspect of pulmonary passive congestion   In some advanced cases, the x-ray films have the appearance of pneumoconiosis, which has led to erroneous diagnosis, as Jimenez has stated

BASAL METABOLISM AND NERVOUS SYSTEM   While the basal metabolism of Andeans is normal according to Hurtado (5f), I have pointed out that, in moderate cases of subacute mountain sickness, there are often lower values which approach or reach normal metabolic rates with acclimatization   There is hyper-

tonia of the vegetative system, as proven by the oculo-cardiac reflex test (Monge, li Pesce, 35a, Aste, 7), during which the Andeans do not feel any distress at all, despite a fall of 50 to 60 in pulse rate In contrast, cases with moderate symptoms of sub acute mountain sickness frequently collapse In normal Andeans Pesce and I were able to give intravenously 3 5 mgm of atropine without clinical symptoms The increased vagal tonus seems to cause bradycardia after exercise The parallel activity of the sympathetic adrenal system (Monge) may explain the tachycardias of sinus origin The hypertonus of the vagal and sympathetic nerves must have some connection with the stability of the vaso motor center and reflex nervous system. Gellhorn's findings on man in experimental anoxia can give an interpretation to the vertigo and the collapsing form of sub-acute mountain sickness In spite of Hurtado s finding of a decreased gastric acidity, observation shows a hunger sensation after moderate exercise Stomach ulcers are aggravated at high altitudes Indigestion and diarrhea are common Certainly some digestive disturbances may be due to the sensitization of the sympathetics by an increase of pH, as Van Liere (19) has suggested Indeed a good deal more investigation of this type is needed (Van Liere)

Clinical observations show that changes in the psychological behavior at high altitudes are very frequent (Barcroft, Encinas), and several psychiatric conditions may result (Monge) McFarland (20) has carried out the most important research on the members of the International Expedition to the Andes By means of psychological tests involving complex mental functions, he found reliable differences from performance at sea level These manifestations are in harmony with the effects observed by the same author in flying or in pneumatic chambers

On the basis of his experiments, Hahn (21) (of the National Institute of Andean Biology) states that it is a mistake to generalize, since some subjects are not affected by altitude All psychological activities are slowed and it is difficult to maintain attention (Ziehen's method) Even a very complicated task, involving eye and hand co-ordination (mirror drawing of Giese), could be carried on in the mildest forms of sub-acute mountain sickness but more time was required Mental training and automatization seem not to be affected He emphasizes that in color naming (McFarland s method), if the verbal expression is substituted with a wordless procedure (Hahn's), the rate of errors is not affected by altitude Regarding Andeans, there are no marked differences in visual reaction time, but lower motor speed values (tapping of Whipple) are found As regards attention, he found clearly lower values than at sea level (Toulouse, Pieron's method—unpublished work)

Blood acid-base balance Reported changes of acid base equilibrium produced by hyperventilation, based on hydrogen ion concentration observations, are not in agreement because researches have been carried out with subjects whose acclimatization had not been entirely determined Some of them evidently suffered with sub-acute mountain sickness Monge pointed out (1928) that adapted people have a higher serum pH value than acclimatized people Dill, Christian

sen and Edwards (22) found in adapted men a tendency for a slight increase in pH, progressive with altitude to about 4 6 km   Above this level the pH diminished but was higher than that of acclimatized residents

Aste reported the same facts in chronic mountain sickness at 3 2 km altitude In general, the blood pH value is found within normal limits with a marked tendency to be on the alkaline side   Aste, as will be seen later, found a greater difference of pH between arterial and venous blood in Andeans than the normal value at sea level in his studies on chronic mountain sickness   Howevc̈r, Dill, Talbot and Consolazio (23) found normal values at a higher altitude   Dill, Talbot and Consolazio showed that in habitable places, both CO_2 and alkaline reserve diminished, but never in sub-acute mountain sickness did the fall reach the lowest values of the residents, according to them, months will be required to reach these values   With very crude methods, V Villa-Garcia (1935) and I (1j) arrived at the same conclusion, measuring the increase during adaptation Since 1928, I have emphasized the fact that, in spite of a minimal p CO_2 and CO_2 content, the blood of Andeans has a higher buffer capacity than that of individuals with sub-acute mountain sickness   At least this capacity is equal to that of the sea level man who has a much greater p CO_2 and CO_2 content   Probably, there must be a readjustment of buffers, the nature of it being unknown, thus the buffer system must be compensated   The increased hemoglobin might account for some of the difference according to Dill, Talbot and Consolazio (23)   They state that there is a deficit of 2 milli-equivalents in the balance of electrolytes, at 5 3 km altitude   However, the problem is not yet solved   Certainly the organism as a whole must find some new regulatory mechanism to stabilize the internal respiration as a balanced mirror of the external environment   The different steps described by Y Henderson, as a consequence of decreased oxygen pressure, have been outlined in sub-acute mountain sickness by Monge (1928) who established the reciprocal relation between the blood alkaline tide due to diminished CO_2 and the elimination of the bases through the urine as measured by pH ammonia, and titratable acidity   A reversible process is found on returning to sea level (Monge, 1b)   I have observed in Andeans (1928–1935) that the shift of pH after moderate exercise is equal to or less than that attained at sea level   In sub-acute mountain sickness it is considerably greater and diminishes with acclimatization, as has been proved by standard exercise performances (Monge, 37)

Andeans show a high capacity for work   "Every few minutes one would appear (Cerro de Pasco, 14,700 ft altitude) carrying a weight on his back of a hundred pounds brought up from 250 feet lower down" (Barcroft)   The reviewer insists upon these facts because the literature of this subject is full of erroneous statements since the time of Jourdanet and Barcroft   The Andean is constructed like an athlete   This means that sub-acute mountain sickness is a disease of temporary or permanent disability, a fatigue disease   As in athletics, acclimatization means fitness for continuous anoxia   Mountain sickness suddenly changes the endurance of a trained individual into an unfit, unable to reach a high pulse rate as at sea level, or to hyperventilate adequately to permit suitable

work of the heart (Christensen, 36)   Certainly the transport of CO_2 the enzymatic activity of the internal milieu in fixing O_2, and a greater cardiac output, must play a most important rôle in the altitude homeostasis, as Dill suggested (24)

Blood   Barcroft (2), Heraud (1k), Hurtado (5a-g), Hurtado and Guzman Barron (5h), Talbot (25), Capdehourat (11) and Aste (unpublished work) have confirmed the high altitude polycythemia found by Viault and have studied the blood morphology and hemoglobin, which do not differ from the situation at sea level (Hall, 26)   I have summarized the most recent observations in table 1

It can be seen that the red cell count, hemoglobin and hematocrit are higher than at sea level   Hurtado has calculated the several blood variables   He pictures the erythrocyte as a cell of greater size with less hemoglobin and oxygen saturation to facilitate the gaseous interchange.   In general, the results need to be revised in relation to ecological surroundings   In sub-acute mountain sickness, Monge has found a higher count of erythrocytes than the normal value at

TABLE 1

CONDITION	AUTHOR	ALTITUDE	R.B.C.	HEMO- GLOBIN	HEMA- TOCRIT R.B.C	M.C.V	M.C.Hb	M.C.Hb CONCENT
		meters	*millions*	*grams per cent*	*per cent*	*cu microns*	*γγ*	*per cent*
Acclimatized	Hurtado	4 500	6 66	15 93	71 1	96 20	24 4	24 9
	Aste	3 200	5 85	17 69	47 9	82 60	30 5	36 9
Subacute moun tain sickness	Talbott	3 660	5 54	17 23	49 6	89 50	31 1	34 7
	Talbott	4 710	5 84	17 90	53 4	91 40	30 6	30 6
Chronic moun tain sickness	Aste	3,200	6 54	20 92	56 2	85 46	32 2	37 2
	Hurtado	4–5,000	8 26	24 70	77 5	94 1	30 3	32 2

the same altitude (3 7 km.)   This may be deduced also from Talbot's data at 2.8 km in the Andes.   Talbot s observations show a progressive increase of the count up to about 5 km altitude but it never attained the level of residents Higher up, at 6 1 km , the count was lower   In general, when the adaptative stage reaches an upper limit and acclimatization does not come, the number is enormously increased   The hemoglobin follows a parallel curve   Talbot suggests that to reach a maximum of hemoglobin months or years may be required Hurtado found in Andeans a blood viscosity of 8 6 ($\pm$ 0 14), a coagulation time of 6 5 ($\pm$ 0 12), a serum bilirubin of 0 7 mgm per cent   Urteaga (unpublished work) has found in 6 cases a bilirubinemia average of 13 7 per cent   direct Van der Bergh 4 per cent, indirect 9 7 per cent.   He considers this increased threshold of bilirubinemia as normal since the excretion bilirubin test gives normal curves, if referred to this higher threshold   He suggests it might be a device to save the pyrrholic nuclei needed in hematopoiesis (Urteago, 27) Palti (28), at 3.2 km in one hundred native Andeans, has found a calcium value of 11 62 ($\pm$ 0 05) mgm , slightly above that of 11 06 ($\pm$ 0 06) at sea level   The

active Ca was higher at the high altitude   In sub-acute mountain sickness, hypercalcemia, returning gradually to normal, was noted in severe cases   Salas (29), in fifty natives at 3 2 km , has found higher blood protein values than at sea level   In sub-acute mountain sickness the proteinemia was still higher   Perhaps his observations did not last long enough to encounter a reverse change San Martin (30) has confirmed in seven individuals the slight blood sugar increase found first by Forbes (31) in sub-acute mountain sickness, and in addition has noted higher values of blood corpuscle sugar compared with sea level values He found 0 71 mgm per cent, instead of 0 59 mgm , in hematocrit value, 0 97 mgm instead of 0 46 per cent   Probably with acclimatization blood sugar may come back to lower levels, as was found by Forbes   There are not enough data to make definite conclusions   I have found the white cell count normal in Andeans but slightly increased in sub-acute mountain sickness   Most impressive are the increased number of reticulocytes and the presence of an enormous number of histiocytes in mild forms of altitude disease   Even in normal men they have been present (Hurtado, Barcroft, Monge, Talbot)

Barcroft's work regarding arterial blood O_2 saturation established that the Andeans had a lower value (80–82) than newcomers (90–92)   Hurtado confirmed this, as did different members of the Chilean International Expedition The most extensive work has been done by Hurtado, who has established particular levels of arterial blood O_2 for different altitudes   Aste, at 3 2 km , has found saturations more like those at sea level (93 5)   Dill, Christensen and Edwards (22) have confirmed Barcroft's view that diffusion alone accounts for the transfer of gases in the alveolar epithelium   Keys, Hall and Guzman Barron (32), working at several progressive altitudes, have stated that the "physiological" deviation curves tend to shift to the left of sea level values until about 4 5 km altitude and to the right at higher stations   "This may or may not be related with the fact that somewhere around this altitude there is not a relationship between O_2 arterial saturation and the well-being of the subjects "   On the contrary, the natives have the lower saturation   So, in order to explain the inability of the body to fix oxygen in mountain sickness, Guzman Barron, Dill, Edwards and Hurtado (33) suggested a disturbance of the oxidation-reduction system   Edwards (34) argued that the problem is one of utilization rather than of transportation and suggested that myoglobin plays an important rôle   In fact, Hurtado, Rotta, Merino and Pons (35) proved this to be true as regards the myoglobin   Since Campbell's research, particular attention has been paid to the oxygen capillary pressure   In discussing acclimatization, I reported higher venous O_2 saturation and pressure in cases of sub-acute mountain sickness as compared to Andeans, as if the tissues had not taken oxygen from the blood (1928) (1a)   Aste (unpublished work), comparing the arterial and venous O_2 saturation of normal and chronic mountain sickness individuals, has found facts in agreement with my previous investigations   Thus he points out in acclimatized men an arterial-venous O_2 difference of 60 5, as compared with only 39 8 in patients   In spite of the fact that there are other factors which can influence the uptake of O_2 from the blood, it is interesting to point out that a characteristic of

mountain sickness is that the O_2 flows by without being taken up by the tissues The same holds true for patients suffering with sub-acute mountain sickness who, on being brought to sea level during the recuperative period, show a diminished O_2 intake   These facts were so impressive that the reviewer could suggest (1928) that "altitude changes the capacity of the tissues to fix O_2 We can suggest that there are unknown substances which bring about acclimatization" (1a)   This opinion still is in harmony with the modern concept of biochemistry (38)

Chronic mountain sickness   The clinical picture resembles fully advanced cases of Polycythemia vera   The patient, at rest, has either an erythrose color, which turns blue on the least effort, or a dark cyanotic color more conspicuous in the face and hands   There is a generalized dilatation of the blood vessels The sclerae are intensely colored by distended capillaries, the eyes being hidden behind edematous and bluish eyelids   The nasal and oral mucosae are wine red The skin of the body is dry, while the forehead and hands are usually covered with sweat   The hands show clubbing of the fingers   The nails appear to be inserted like watch crystals   Epistaxis is frequent   hoarseness and aphonia are often noted   The thorax is enlarged—of the emphysematous type   The patient feels extremely weak, has a tendency to sleep and is frequently found in a state of drowsiness   Sometimes he falls into coma for two or three hours   Spells of dizziness and faintness occur   On occasions there are crises of vomiting Constipation alternates with diarrhea   Dyspnea is permanent, bronchitis is frequent   There are recurring congestive processes of the lungs with hemoptysis and low fever, which disappear when the patient goes down to sea level   Cardiac disorders are not found for a long time, but right heart insufficiency supervenes with the progress of the disease   Cardiac pains of anginal distribution appear in some cases   In one case, simultaneous with angor, the vision grew cloudy and the patient became unconscious and fell   Hyperthermia very rarely occurs Temporary blindness (Dammert) and temporary deafness are not infrequent The liver is moderately enlarged   Enlargement of the spleen has been found in 12 per cent of the cases

Algesias and paresthesias are a common complaint.   Excruciating pains in the extremities and pain in the lumbar region or in the joints   particularly in the tendon attachments at joint cavities, are present   The pain sometimes immobilizes the patient during several days or weeks, only to disappear spontaneously or when descending to a lower altitude   Bleeding improves this condition Some of these symptoms which I described (1928) are similar to those found by Armstrong in aviators   There may be violent cephalalgia, subsiding after lumbar puncture   Paresthesias are varied in type and localization   unpleasant sensations of heat in the face or of hot water being poured on the back   One patient had the sensation of the loss of one hand   Formication in the feet and the sensation of being pricked by pins are frequent

In cases of severe involvement it is possible to find marked disturbance in the behavior and memory of the patients—the entire psychic personality appearing altered   Nervous exhaustion is very common   The patients complain of sexual frigidity

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an eosinophilia Certainly there is established at high altitude a new equilibrium between the increased hematopoiesis and the increased destruction of red cells

The characteristic feature that gives this disease its personality is the fact that all troubles and ills disappear when the patient is brought to a lower altitude or, of course, to sea level This undoubtedly is due to a single cause anoxemia Usually after a stay at sea level, the patient can return to the altitude and live there for some time without great discomfort As time goes on, however, the sea level cure fades and asphyxial disorders conducive to immediate death in coma and acidosis may occur as soon as the patient reaches high altitude Most of the patients become acclimatized temporarily at a lower altitude, but at times they have to go down to sea level

I have described several varieties of chronic mountain sickness, according to the predominance of oxygen want in certain tissues or organs I shall enumerate them briefly In the congested cerebral form (1f) appear (1936) spasms of pain ful headache, fullness of the head, photophobia, capillary ocular injection, blur-

TABLE 2

BLOOD	CONDITION	R.B.C.	HEMO-GLOBIN	HEMA-TOCRIT R.B.C.	M.C.V	M.C.Hb	M.C.Hb CONCENT
		millions	grams per cent	per cent	cu. microns	γγ	per cent
Venous	Acclimatized	5 85	17 69	47 9	82 6	30 5	36 9
	Unacclimatized (chronic moun sickness)	6 54	20 92	56 2	86 4	32 2	37 2
Arterial	Acclimatized	6 14	17 50	47 3	77 5	28 7	36 9
	Unacclimatized (chronic moun sickness)	6 21	20 21	57 4	92 5	32 7	35 3

ring of vision or deafness, scotoma, lacrimation, vertigo, dizziness, general sweating and vomiting, intense cyanosis, bradycardia, drowsiness and uncon sciousness The spinal fluid pressure is enormously increased Arellano (6) has established that repeated lumbar punctures relieve the patient Frequently spontaneous epistaxis ends the crisis Neurological forms, with monoplegia or hemiplegia, sometimes appear In one case, after several months of moderate erythremic symptoms, the patient suddenly developed a modest degree of palsy of the right arm and aphasia lasting several minutes Every morning for eight days the trouble returned On coming down to sea level the symptoms disap peared Two months later the patient returned to high altitude but after a stay of three weeks the troubles reappeared and he was obliged to give up his work Now he is entirely normal after two years at sea level Collapsing forms are frequent and usually are associated with a syndrome of hyperventilation and tetany In one case of moderate erythremia at 4 6 km , two attacks of tetany occurred at an interval of several years, provoked by a light bronchial inflamma

tion   The condition was so severe that the patient, in status tetania, was transferred to a lower altitude (2 8 km) and the symptoms disappeared during the trip

Cardiac, digestive and pulmonary emphysematous forms are frequent   I have observed one case of erythremia with crises of intense cephalalgia (once relieved by lumbar puncture) accompanied by albuminuria, all of which disappeared when the patient came to sea level   The symptoms and the albuminuria recurred again at the high altitude   At sea level during seven years the patient remained normal   Recently he returned to high altitude and developed a syndrome of high arterial pressure, without renal insufficiency, which was cured on returning to sea level

Mental forms are common (Monge, 1g)   In a recent case a change of the personality was surprising   The patient found himself unable to work, he saw everything wrong, he was afraid to meet his employees   Sometimes he got up at night and pretended to work   He realized that there was nothing to do but he went just the same   Finally he conceived the idea of committing suicide   He was immediately relieved on being brought to sea level   The mental disturbances came back when he returned to the high altitude   At sea level he now enjoys a permanent cure

When one considers the clinical syndromes which lead to polycythemia, a clear division into two groups may be established   To the first group belong the lung and cardio-arterial processes which act mechanically on the permeability of the respiratory epithelium   Its symptomatology, besides polycythemia and changes of the acid-base equilibrium, is distinctly pulmonary   In the second group there is polycythemia and a definite clinical syndrome—the so-called erythremic syndrome (Vaquez, Osler, Monge) with its ubiquitous pathology never found in the first group   These two diseases of equal nosographic symptomatology, polycythemia vera and chronic mountain sickness, have different pathogenesis the cause of the second is clear—anoxemia   No one knows the cause of polycythemia vera   After my first paper (1935), I was inclined to accept the identity of both because there is a gradient of frequency of cases from the high altitude "inhabited" places to the lower "habitable" places and finally to sea level   Thus I do not see any reason to discard the possibility of the loss of acclimatization at sea level   It would remain to be demonstrated that oxygen therapy relieves selected cases of Vaquez disease at sea level   Unfortunately the therapy done in compressed chambers is not conclusive   Several months, at least, are needed to have a steady amelioration at sea level   Hypertension and enlarged spleen may be found in chronic mountain sickness

As a hypothesis of pathogenesis I suggested (1928) that impermeability of the lungs is due to a low diffusion coefficient of O_2, as can be deduced from Harrop's work (39)   However no proof has been given   Hurtado (5d) in his last paper stresses the importance of lung fibro-sclerotic changes due to anoxemia, increased arterial pulmonary pressure and high viscosity   He thinks that morphology has been unduly neglected   Structural changes of the lung alone, as in

pulmonary sclerosis, would not explain the universal cellular pathology which occurs in chronic mountain sickness So it may be a primary chemical dysfunction If the hypothesis of Guzman Barron, Dill, Edwards and Hurtado concerning the alteration of the oxidation reduction systems due to low O_2 pressure, which is supported by the findings of Monge (1928) and Aste (1940) regarding O_2 not taken by the tissues, is accepted it must hold for lung tissue metabolism Certainly the lining of the respiratory cells which regulates O_2 passage from the alveolar air to the blood must be included in the same enzymatic dysfunction Besides, this hypothesis must be extended to the mechanism which regulates the transport and elimination of CO_2 for carbonic anhydrase according to Meldrum and Roughton plays a decisive rôle in the output of CO_2 from bicarbonate in the lungs Dysfunction would mean disturbance of CO_2 elimination which always occurs as soon as mountain sickness begins There is not any fact suggesting a deficiency of mineral traces Normal enzymatic equilibrium explains the whole process of the acclimatized organism integrated by hormonal and nervous devices Its failure would mean chronic mountain sickness either ubiquitous or elective as it happens to be, in the case of lesions of the germinal cells

The lasting treatment is the cure at sea level I have tried x ray therapy to shorten the time of recuperation

Inapparent chronic mountain sickness and the physiology of reproduction at the high plateaus It is a very well known fact in South America that high altitude exercises a deleterious influence on fertility Clinical observations prove it in man and in animals (Monge 40f) I have described cases of fertile couples at sea level who were infertile at high altitude In one case the infertility of the male was demonstrated Carvallo (personal communication) found two cases of sea level men coming from high altitude in which no spermatozoa could be found It is a common fact that adapted pregnant women often come down to the coast to be delivered because miscarriages and sometimes sudden death of the new born happen at the heights In animal breeding I have pointed out (1940) some interesting facts Eggs from sea level do not hatch at high altitude (Humboldt, Agazzoti) A couple of dogs brought to Lassa (Thibet) never reproduced according to Cutting (41) Sterility in rabbits, horses, cattle and cats has also been found

It is interesting to recall Father Calancha's observation (1039) that the Spanish conquerors at 14,000 feet (Potosi Bolivia) did not have offspring until fifty-eight years after the city was founded In 1535, the capital of Peru was transferred from Jauja (11,500 ft) to Lima (sea level) because the horses, fowl and pigs did not reproduce, as is stated in the act of foundation of Lima Father Cobo (1605) established Mendelian anticipations in saying "The Indians are stronger and multiply enormously while the majority of the children of Spanish parents born in such climates do not live   the climate's influence is visible in the half breed and quarter breed,   the more Indian blood, the better they grow (40a) ' On the other hand, the birth rate of the Andeans is equal to that

of the sea level people   I quote from the last census of Peru, the following figures through the courtesy of Dr Arca Parro

	ALTITUDE		
	0–2 km.	2–3 km	3–4 4 kgm
Population	2,251,718	1,596,004	1,742,229
Birth rate %	28	26	26

Thus the physiology of reproduction in the high plateaus is normal in regard to the birth rate of the Andeans

Research in the physiology of reproduction related to subacute, chronic, and particularly inapparent chronic mountain sickness has been conducted by Monge and associates (40b)   Monge and Mori-Chavez (40e), on examining cats sent to 4 4 km altitude, found that the testes were devoid of the germinal epithelium, Sertoli cells replacing spermatogonia   Even basal spermatogonia had disappeared   The Leydig cells showed a noticeable increase   In some rabbits they found that the majority of the seminiferous tubules had only the basal line of spermatogonia and Sertoli cells   In others, there was a second row of spermatocytes but neither spermatocides nor spermatozoa   In some rabbits the process of recovering was visible

The research in sheep planned by Monge (40c) and conducted by San Martin (42) is very promising   Sheep acclimatized at high altitude since the seventeenth century have 100 per cent fertility   The fertility comes down to 30 per cent when pure-blooded imported rams, from sea level, are used for cross breeding   With progressive adaptation, fertility can be raised to 60 per cent to 70 per cent   But in order to attain this result 4 to 7 rams per hundred are used instead of the one or two at sea level   So there is in general a very high percentage (40–50 per cent) of infertility which, according to current opinion, is due to the ewes being infertile ("machorras")   This percentage, in my opinion, could be the statistical result of ewes mated with rams of unproven fertility   Actually when the rams are brought to a lower altitude they recover their fertility   From the research now in progress, two facts deserve special mention   In a group of 67 supposedly selected acclimatized rams, the variation of semen pH was 5 6 to 8 5, which indicates that the pH regulatory system fails at high altitude   The motility was diminished and the sperm was not suited for insemination   Only in 23 was the pH within normal range 6 2 to 6 8   Recently, with the aid of San Martin, a study has been made of 2 rams selected as sires in Chile (sea level altitude) and brought to Huancayo (3 2 km altitude)   Their food was selected, both suffered from mountain sickness during the first week   Then they were apparently normal   One, after eight months, does not have spermatozoa but has an exaggerated sexual desire   The other, after an initial stage of reduced spermatogenesis (twelve million germinal cells instead of a thousand million), high pH (8 5), and absence of motility, has recovered   However, he has very low sexual desire   Easly, on the other hand (unpublished

work), tested a group of rams recently brought to an altitude of 4 km. and found 40 per cent of them fertile

By selecting normal semen and artificial insemination, it was possible to get 86 4 per cent pregnancy in the supposedly infertile ewes ("machorras") (San Martín)   Thus was proved the infertility of the males and the fertility of the females ("machorras") (1941)   The emphasis given to this point is explainable because it concerns eleven million sheep in Peru alone [2]   In a field experiment at the altitude of 4 km with only one selected ram, seven hundred ewes were mated with a resulting pregnancy of 68.8 per cent   The control group, seven hundred ewes mated with 49 rams as usual in the high plateaus and under natural conditions, gave only 60 per cent pregnancy   So animal races can be created through scientific discrimination

Here I enter on particularly interesting ground   race pathology, as a function of inapparent clinical disturbances   Chronic mountain sickness cannot be judged only by a general appearance or by an outstanding clinical symptomatology   Its influence may be extended electively to the germinal cells   In high altitudes men both physically and mentally, might seem to be at their best but at times they are sterile   In animals the same thing occurs.   Even though the sexual desire is manifest, the observer may be misled   It is not an exaggeration to stress the gravity of these findings in regard to the problem of population and the possibilities of human and animal races not suited for acclimatization   One is obliged to accept the fact that the autochthonous Andean man belongs to a climatophysiological variety of human race which has adequate power of reproduction in an atmosphere of permanent anoxia.   This may help to explain the attachment which that man has for his altitude environment, the ailments suffered by him in the traumatic climate of the lowlands (lowland sickness) and the urge to return home that has made of him a nomad, and finally the old Inca mandate that permitted mass population displacements only to similar climates—a factor still active in the annual work migrations   Certainly, the Incas knew more about the influence of the high plateaus upon the individuals and the behavior of the autochthonous societies of America than do men of today (Monge)   Acclimatization has a biological determinism on the individual, the race, the societies and the history of South America (Monge, 40d)   The unfit are eliminated through a process of natural selection (chronic mountain sickness), either visible or inapparent

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THE RÔLE OF THE LIPIDS IN ATHEROSCLEROSIS

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Atherosclerosis of the aorta and its branches, by far the commonest vascular disease, ranks high among the causes of natural death in man. Although the records and observations of atherosclerosis in man and animals have great antiquity, no generally acceptable cause for the lesions has been established. The tissue changes of atherosclerosis are regarded by some as part of an ageing process of the vessels, but according to the opinion of others the lesions are caused by lipid infiltrations of the intima and result from disorders of lipid metabolism.

Deposits of lipids, the formation of fibrous plaques and atheromas, and the deposition of calcium in the intima of the aorta and its branches are the dominant changes of atherosclerosis. The initial gross manifestations of the disorder are fatty streaks or small nodules in the root, arch and posterior wall of the aorta, especially about the ostiums of the intercostal arteries and other branches. Growths of fibrous tissue in these fatty deposits form larger discrete and confluent lesions. Later, with necrosis, the centers soften and become masses of soft lipid material and tissue debris, the atheromas. When the lining edge of the atheroma breaks, the lesion becomes an atheromatous ulcer. Simultaneously calcification may occur, and later even bone tissues may form. Injury of the lining of the blood vessels opposite these lesions favors thrombosis which increases the gravity of the atherosclerotic lesions. Complete descriptions of the gross and microscopic changes in aortic and arterial tissues with atherosclerosis have been published by Marchand (44), Duff (18), Leary (39, 40), Aschoff (4, 5) and Frey (21).

Many pathologists, among them Virchow (68), Sanders (58), Beitzke (9) and Wells (74) have stated that the development of atherosclerosis depends upon focal injuries or retrogressive changes of the media, commonly attributed to an ageing process, the intima lesions, accordingly, being of secondary significance. Another view favored by Marchand (44), Lubarsch (9), Aschoff (4, 5), Anitschkow (2, 3), Leary (39, 40) and others has emphasized the importance of the lipid infiltrations into the intima. The lipid deposits, thus, are primary, initiate the other changes of the vessel walls, and atherosclerosis is a specific disease in which ageing of the tissues is only a contributing factor.

Morphologic studies and chemical analyses of human and animal aortas have attempted to establish the significance of the lipids in atherosclerosis. The morphologic approach has demonstrated the presence of lipids, especially cholesterol, in the tissue lesions. Chemical analyses have revealed the composi

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tion of these lipid deposits and have provided data for comparisons with the composition of lipids in other tissues of the body   The results of these chemical analyses also set apart the changes of atherosclerosis from those developing with senescence of tissues   Our review is limited to these chemical phases of atherosclerosis

CHANGES IN THE CHEMICAL COMPOSITION OF THE AORTA WITH AGE   Atherosclerosis of the aorta and arteries is associated with an increased lipid content of the tissues   The chemical analyses demonstrating this increase are summarized by Wells (74)   Because these analyses of entire aortas included both the normal and the atherosclerotic tissues, no distinction was made between the chemical changes occurring with the disease and those due to senescence   Weinhouse and Hirsch (71), in a study of these problems, quantitatively analyzed human aortas with and without atherosclerosis   To avoid the possibility of including pathologic tissues, only media, carefully dissected from the intima and adventitia, was used   The absence of retrogressive changes in the media was verified by microscopic examination

TABLE 1

Variation in composition of media by age groups

AGE GROUP	MOISTURE*	LIPID EXTRACT†	CHOLESTEROL†			PHOSPHATIDE†			GALAC TOSIDE†	CALCIUM†
			Free	Total	Ester	Ether soluble	Ether insoluble	Total†		
0–40	68 4	6 42	1 05	1 78	1 24	2 13	0 22	2 21	0 59	0 33
41–60	71 8	8 31	1 36	2 23	1 44	1 25	1 53	2 70	1 09	1 10
61–84	70 3	10 57	1 87	3 84	3 30	1 29	1 59	2 91	0 95	2 57

* The average content is given as percentage of wet tissue
† The average content is given as percentage of dry tissue

Lipids   A progressive increase in all lipid constituents of the media with age was observed (table 1)   The free cholesterol in the highest age group was almost double the average value for the lowest age group   The cholesterol combined as ester in the highest age group almost tripled the amount in the lowest age group   The phospholipids as a whole increased only slightly with age   Within this class, however, there were relatively great changes   In the aortas of youths, ether-soluble phospholipids (lecithin and cephalin) preponderated, in the older aortas, ether-insoluble phospholipids (sphingomyelin) constituted the major portion of the phospholipid   The increase in the sphingomyelin fraction of the aorta from the lowest to the highest age group was great, from 0 22 to 1 6 per cent of the dry tissue   An increase with advancing age was observed also in the galactoside content, an observation in agreement with analyses by Kimmelstiel (32)

Calcium   The calcium content increased greatly with age from a low value of 0 33 per cent to 2 57 per cent in the highest age-group   The increase, occurring without evidence of tissue degeneration apparently is a normal process without causal relation to pathological calcification of the intima   This is

demonstrated clearly by a comparison of the calcium content of the media with the corresponding intima   In the middle age-group, for example, six medias contained approximately equal quantities of calcium   Two had no intimal changes, three had moderate atherosclerosis and the other had marked athero sclerosis and calcification   The lack of correlation between intimal and medial calcification was more obvious in the highest age-group   Of the two aortas in which the media contained the lowest calcium content, the intima was markedly calcified   In one aorta, where the media had the highest calcium content, the intima had the slightest atherosclerosis, without calcification   A similar increase of cholesterol and of calcium with advancing age has been re- ported by Bürger (11) not only in the aortas of man, but also of horses and cattle

Moisture   Dehydration of the tissue colloids in the media has been proposed as an important factor of atherosclerosis   This opinion is based upon the observations by Bürger and Schlomka (12) that tissues such as costal cartilage, cornea and lens lose water progressively with age   The loss in water, accord ingly, decreases the capacity of the tissues to hold substances in solution, where- by the least soluble constituents, namely, cholesterol and calcium precipitate However, table 1 revealed no significant change in the moisture content of the media with increase in age   This agrees with the results reported by Bürger (11)

Summarizing the chemical changes of the media with age   there is a significant increase in the free and total cholesterol phospholipids, galactosides and gly cer- ides as the tissues become older   There is a marked increase in the calcium but no change in the moisture content   All of these changes have no obvious rela tionship to the severity of the intimal lesions   Chemical evidence   therefore, does not support the opinion that atherosclerosis occurs as a result of changes in the media

Macroscopically and microscopically normal intima tissue is relatively rare, consequently too few analyses were carried out for conclusions regarding the effect of age on chemical changes in the intima   Tissues from five normal in timas were analyzed   In general they had a higher lipid content and lower calcium content than the corresponding media tissue   The average values for intimal constituents calculated as per cent of dry tissue were free cholesterol 2.26 per cent, total cholesterol, 5 92 phospholipids 2 05, galactosides, 0 08 total lipids, 14 4, and calcium 0 23

CHANGES IN THE CHEMICAL COMPOSITION OF THE AORTA WITH ATHEROSCLERO- SIS   *Cholesterol*   The first quantitative determinations of cholesterol in athero sclerotic aortas were reported by Windaus (77)   He found in two normal aortas 0 119 and 0 107 per cent free cholesterol, and 0 032 and 0 047 per cent esterified cholesterol   In two atheromatous aortas, the values were 0 741 and 0 673 for the free cholesterol and 1 043 and 0 792 for ester cholesterol   Thus, the athero matous tissues contained six to seven times as much free cholesterol and about twenty times as much esterified cholesterol as normal tissues

The next significant contribution to the chemistry of atherosclerosis was by Schönheimer (59, 60) who made a systematic study of the effect of age and

atherosclerosis on the cholesterol content of the aorta   In the normal aortas of youths, he found only small quantities of lipids, with increasing age and increasing atherosclerosis there was a marked rise in the lipid content   In a series of these aortas the lipid content ranged from 0 2 to 1 8 grams   The proportion of cholesterol in the extract was relatively constant, varying only from 23 to 32 per cent of the total lipids despite the great variation in the amount of total lipids   The esterified cholesterol, however, increased with the rise in total lipids, amounting to 57 per cent of the total lipids in the atherosclerotic aortas On the basis of these results, Schönheimer concluded that the original lipids of the aorta are augmented, in the development of atherosclerosis, by a lipid mixture containing a constant proportion of free and total cholesterol   Thus analytical evidence corroborated the view that the lipids of the blood infiltrate and are deposited in the intima

Quantitative analyses of human aortas by Kimmelstiel (32) demonstrated a proportional increase of cholesterol, phosphatides and galactosides until atheromas formed   The relations then changed and cholesterol increased rapidly in comparison with the other lipids   Atherosclerotic aortas of non-diabetics according to Lehnherr (41) contained increased amounts of cholesterol, phospholipids and fatty acids, a greater proportion of total cholesterol in the lipid fraction, and larger quantities of calcium and phosphorus with a diminished Ca/P ratio   The lipid deposits in the atherosclerotic aortas of diabetics were greater, but the quantitative relations of the fractions were similar   The analyses of the "fat" content in 500 aortas by Rosenthal (57) confirmed those of previous reports disclosing a larger total lipid content in the tissues of aortas with atherosclerosis   He made no determinations of the individual lipids

These analyses of entire aortas gave valuable information but did not differentiate between the chemical changes which occur with advancing age and those due to the disease   This was emphasized by Meeker and Jobling (45) who obtained a better understanding of the chemical changes occurring with the atherosclerosis of the aorta by analyzing the individual lesions, separated from the other tissue, and by comparing the values with those of the normal tissues

In the normal tissues, 40 per cent of the lipids was cholesterol, of which 28 4 per cent was esterified and 12 6 per cent free, 16 per cent was phospholipids and about 20 per cent was unidentified   As the lesions increased in severity, the proportion of cholesterol increased greatly, the increase being due almost entirely to free cholesterol   The ratio of free to bound cholesterol increased from 0 43 in the normal tissue to 1 04 in the most advanced lesions   A later study, by Zeek (78), substantiated these results

As a part of a chemical study of atherosclerosis in the human aorta, Weinhouse and Hirsch (71) separated atherosclerotic lesions of the intima from the surrounding tissues, determined the calcium, lipid and moisture content, and compared these values with those of the normal intima tissue   The atherosclerotic lesions were separated carefully from the media and surrounding intima They were classified as fatty plaques, fibrous plaques, calcified plaques, and atheromatous ulcers   The first was the nodular or streaked yellow lesion in

which the lipid material was mainly intra-cellular, and the intima thickened slightly but not scarred appreciably The second form was the raised nodule or diffuse plaque of tough fibrous tissue with a pearly luster, necrosis, and confluence of lipids The third form consisted of brittle calcified plaques with soft necrotic centers The atheromas were ruptured or about to rupture and contained, in addition to soft lipid material, blood clots and calcium deposits The results of these analyses are summarized for comparison with those of normal intima in table 2

They demonstrated a progressive increase in the lipid content of the diseased tissues The relatively low lipid content of the calcified lesions was due to the great density of the calcium salts contained therein

Cholesterol As the lesions progressed, the proportion of the cholesterol in the lipid extract rose from 14 2 per cent in the normal intima to 27 per cent in the late stages The proportion of cholesterol esters increased less rapidly and in

TABLE 2

Average values of constituents of intimal lesions

TYPE OF LESION	MOIS TURE	LIPID EX TRACT†	CHOLESTEROL‡		PHOSPHATIDE‡			FATTY ACIDS‡		
			Free	Ester	Ether soluble	Ether insol- uble	Total	Galac toside	Neutral fat, etc.	Cal- cium†
Normal intima	71 6	14 4	14 2	38 6	13 7	6 4	20 1	8 0	19 1	0 23
Early fatty plaques	67 5	25 9	16 2	38 5	10 8	8 2	19 0	5 8	20 5	0 86
Fibrous plaques	66 5	27 2	18 1	47 5	5 9	9 0	14 9	4 5	15 0	3 04
Calcified tissues	38 6	12 8	21 9	47 2	3 9	9 3	13 2	4 0	13 1	24 3
Atheromatous ulcers	60 8	36 0	27 2	42 1	5 8	10 2	16 0	4 3	10 4	10 1

The content is given as percentage of wet tissue
† The content is given as percentage of dry tissue
‡ The content is given as percentage of total lipid

the lesions with the most extensive necrosis their amount was lower than in the intermediate stages In agreement with Meeker and Jobling (45), the ratio of combined to free cholesterol declined in the advanced lesions of atherosclerosis, despite an increase in both substances

Phospholipids There was a slight decrease in the total phospholipids from about 20 per cent in the normal intima to about 13 per cent in the calcified lesions The constitution of this fraction, however, changed greatly with advancing severity of the atherosclerosis In the normal intima, there was approximately twice as much of the ether soluble phospholipids (lecithin and cephalin) as ether insoluble phospholipids in the severely diseased tissues, the proportions were reversed

Other lipids Free and combined cholesterol and phospholipids together comprised from 60 to 80 per cent of the total lipids in atheromatous lesions The remainder consisted of glycerides, fatty acids, and small, though measurable quantities of a reducing lipid substance, presumably galactoside

Calcium   The normal intima contained much smaller amounts of calcium than the media   In five samples of such tissues, the calcium content averaged 0 23 per cent   An increase was observed even in the early plaques, and as the lesion developed, deposition of calcium proceeded rapidly even before visible hardening had occurred   In the grossly calcified lesions the calcium content varied from 17 to 37 per cent, indicating that almost complete replacement of the normal tissue structure by lipids and mineral matter had occurred

FACTORS IN THE DEPOSITION OF LIPIDS   *Origin of the lipids*   Determinations of the composition of the lipids in the intima and in atherosclerotic lesions have added considerable support to the infiltration theory   If the deposits in atherosclerosis of the aorta arise by infiltration of the plasma lipids, the lipid composition of the fatty deposits, with little or no necrosis, should approximate the composition of the lipids in the blood plasma   The most comprehensive study of the lipid composition of human plasma is by Page, Kirk, Lewis, Thompson and Van Slyke (50)   They determined free and total cholesterol and phospholipid in blood plasma of normal men of ages from 20 to 101 years   The analyses,

TABLE 3

Comparison of lipid composition of blood plasma and arterial tissues*

	BLOOD PLASMA	INTIMA	EARLY PLAQUES	MEDIA
Free cholesterol	14 1	14 2	16 2	17 3
Cholesterol esters	38 3	38 6	38 5	16 7
Phospholipids	22 8	20 1	19 0	34 1
Neutral fat, etc	23 3	27 1	26 3	31 9

* The values are given as percentage of total lipid

made according to the gasometric methods of Van Slyke and co-workers, are the best figures available at present   As cerebrosides were not determined by these authors, this fraction has been included in the neutral fat fraction of the figures reported by Weinhouse and Hirsch (71) to provide a better basis of comparison between the two sets of values   Table 3 demonstrates a striking agreement between these analyses, despite the differences in procedures

The close agreement in composition between lipid extracts from the plasma and the normal intima and the wide differences between the lipid extracts from the latter and the media indicate that the lipids of the intima originate in the plasma rather than in the protoplasm of the cells   The comparison also supports the validity of Anitschkow's opinion that the intima is freely permeable to the lipids of the plasma and that the intima has no selective action on these lipids   The close agreement between the composition of the extracts from the normal intima and that of the early fatty deposits suggests further that these deposits arise through non-specific deposition of the plasma lipids   The changes in lipid composition which occur in the late stages of the lesions may result from several processes   These are phagocytosis and other reactions against a foreign sub-

stance, necrosis resulting from altered nutrition, or chemical changes of the lipid substance deposited in the tissues   These processes, however, are secondary to the initial deposition of the lipids

The lipids of the human blood   Page, Kirk, Lewis, Thompson and Van Slyke (50) observed no regular important changes in any lipid fraction of the blood from youth to old age   Their statistical analyses indicated no greater difference between age classes than would be expected from the variability with individuals of similar age   When the total lipids in the plasma increased, the free and ester cholesterol, the phosphatids and the neutral fats participated in this increase, but not to the same extent   Free cholesterol changed the least and neutral fat the most

The concentration of cholesterol in the serum of normal human beings of both sexes averages about 200 mgm with a range of from 100 to 400 mgm per 100 cc (70)   Except for a pronounced rise during childhood, the cholesterol level remains constant   According to the reports of many investigators, during adult life the cholesterol level of the blood tends to remain constant and is not influenced by environmental conditions or living habits   Long periods of starvation or cholesterol deprivation on the one hand, or overfeeding with cholesterol on the other hand, produce little if any changes in the blood cholesterol   When great changes have been observed in normal individuals the amounts of cholesterol administered have been so large as to be unphysiologic   The changes in the blood lipids in alimentary lipemia are almost solely in the glyceride fraction which may vary widely under normal circumstances   Apparently in man, hypercholesteremia does not develop from the ingestion of moderate quantities of cholesterol

The blood lipids in human atherosclerosis   The relationship between hyperlipemia and atherosclerosis in man is obscure   The influence of the amount and distribution of the blood lipids has been studied extensively, but the results are controversial   Since atherosclerosis develops without definite symptoms, determination of its presence and extent is difficult by physical examination   Therefore the relationship of blood lipid levels to this disease can be inferred only in studies of the blood lipids of patients with hypertension, angina pectoris and other diseases closely associated with atherosclerosis

Mjassnikow (46) observed elevated levels of the blood cholesterol in angina pectoris, the values ranging from 190 to 440 mgm per cent compared with 120 to 170 mgm per cent for normal subjects   Davis, Stern and Lesnick (15) likewise reported blood lipid levels frequently elevated in patients with angina pectoris   About 60 per cent had total blood cholesterol levels over 250 mgm per cent, whereas only 20 per cent of normal subjects had values this high   Values higher than normal for the blood phospholipids and fatty acids also were observed   In seventy three patients with arteriosclerosis obliterans, unassociated with diabetes or hypothyroidism, Barker (7) found the mean value for blood cholesterol to be 263 mgm. per cent, compared with 218 mgm for normal subjects   Poindexter and Bruger (54) observed average values of 250 mgm

per cent for the blood cholesterol in twenty-four patients with arteriosclerosis and in nineteen with hypertension and arteriosclerosis, as compared with 195 mgm per cent in a normal group

Other investigators, however, have reported normal levels of the blood cholesterol in clinical diseases associated with arteriosclerosis   Andes, Kampmeier and Adams (1) observed no difference in the cholesterol level of the blood in normal and arteriosclerotic negroes   Page, Kirk and Van Slyke (51), in sixteen patients with hypertension uncomplicated by nephritis, found normal blood lipid levels   In no instance were the free and total cholesterol, phospholipids and total lipids outside the range of normal values, nor were the mean and standard deviations for these lipids different from those in normal subjects   Elliot and Nuzum (20) found no significant elevation of blood cholesterol in fifty-three patients with hypertension and concluded that there was no correlation between the severity of clinical arteriosclerosis and the level of the blood cholesterol

Probably the best study is by Landé and Sperry (37)   Recognizing the difficulties in measuring the extent of arteriosclerosis by clinical methods, they determined the blood cholesterol, postmortem, of persons dying suddenly from violence   After comparing these values with the degree of atherosclerosis, and eliminating those complicated by infection or organic disease, they concluded that no relationship existed between the blood cholesterol level and the severity of atherosclerosis

Apparently, many if not most cases of atherosclerosis are unaccompanied by hypercholesteremia   Although the influence of the blood lipids in the development of this disease has not been disclosed, there seems to be no doubt that the disease may develop without obvious abnormality of the blood lipids

In addition to the results of experimental atherosclerosis in rabbits to be discussed later, clinical evidence indicates that hyperlipemia is a factor in atherosclerosis   The close association between high blood cholesterol levels and atherosclerosis in diabetes has been discussed by Gibbs, Buckner and Bloor (22), White (76), Dragstedt, Clark, Julian and Vermeulen (17), and Rabinowitsch (55)   The high incidence of atherosclerosis in systemic xanthomatosis, associated with hypercholesterolemia, has been reported by Thannhauser (63), Montgomery and Osterberg (47), and Müller (48)   The atherosclerotic tendency among diabetics, according to Duff (18), may be due not necessarily to hypercholesteremia, but to other profound metabolic disturbances in this disease   This opinion receives support from the recent discovery of a pancreatic hormone, lipocaic, the absence of which causes the deposition of lipids in the liver (16, 17)   Perhaps the deposition of lipids in atherosclerosis also may be the result of a hormonal deficiency, although experimental investigation of this possibility has yielded contradictory results   Thus, Huber, Brown and Casey (30) reported that lipocaic prevented the rise in the blood cholesterol and the development of atherosclerosis which otherwise occurred regularly in rabbits fed cholesterol   Dragstedt and his co-workers (66), however, were unable to confirm this   Data are also conflicting with regard to the influence of the lipotropic factor, choline, in experimental atherosclerosis   Himsworth (25) and Baumann and Rusch (8)

observed no effect, but Steiner (62) found that choline inhibited the formation of atherosclerotic lesions and hastened their removal   The presence or absence of such lipotropic factors may explain why the basic diet is important in the development of experimental cholesterol atherosclerosis

Physical distribution of the lipids in the blood   Although particles of lipid from 0.5 to 1 micron diameter in the blood plasma, according to Bloor (10), are lipid in transport, the factors concerned with this physical distribution of the lipids in the blood plasma have not been considered in atherosclerosis   Much of the lipid absorbed from the intestine reaches the blood in this form and is removed and stored as fat   When the lipid particles accumulate in the blood, lipemia results.   Ludlum, Taft and Nugent (42) believed from their studies of the tiny particles of lipid in the blood that the particles are stabilized by a protein film because the first zone of aggregation of the particles occurred at a pH range of 4 7 to 5.3, approximately the iso-electric points of albumin and globulin, and the droplets coalesced when the acidity was sufficient to precipitate the protein and destroy the film   The membrane about globules of fat in milk, according to Palmer and Wiese (52), is a mixture of protein and phospholipid   Churning of the milk removed the membrane   Hirsch and Weinhouse (29) injected emulsified cholesteryl oleate intravenously into rabbits to determine, if possible, its localization in the tissues   The particles of emulsified cholesteryl oleate ranged in size to considerably less than the diameter of erythrocytes   Although large amounts of the oleate were injected, the free and ester cholesterol content of the serum within a few minutes after an injection was not changed appreciably   The injected cholesteryl oleate, apparently, was removed rapidly from the blood   Histological examination demonstrated large amounts of particulate lipid material in the tissue phagocytes   The fat depots of a rabbit injected repeatedly with emulsified cholesteryl oleate stained with scarlet red were colored with the dye and the tissue phagocytes were laden with large quantities of the stained cholesteryl oleate   These experiments demonstrated that particles of lipid material are removed from the blood by tissue phagocytes like other particulate substances   Hirsch and Weinhouse (29) noted that where the fine emulsion of the cholesteryl oleate had broken and larger masses of the lipid had lodged in the tissues, phagocytosis was supplemented by growths of foreign body granulation tissues   The degree of dispersion of the lipid material, accordingly, seemed important in determining how the tissues utilized or disposed of the lipid material, a principle harmonious with tissue reactions toward particulate material of other chemical composition

Site of the lipid deposits in the aorta   Opinions differ as to whether the lipid deposits of the intima are intracellular, primarily, and later extracellular with necrosis of the cells, or are extracellular at first, and then intracellular   Klotz (36) believed that the lipid material of the small fatty deposits in the intima of the aortas of the young at first is chiefly in large phagocytes   Later, the lipid droplets appeared outside of the cells, especially along the inner elastic fibers   Although Klotz considered the fat droplets free in the tissues as having been derived from disintegrated phagocytes, Wells (72) postulated that the fat drop-

lets, at first, may be free in the injured tissues and secondarily are phagocytized by the cells   Duguid (19), he stated, believed the latter more probable   Leary (40), thought the lipophages developed from the free mononuclear cells of the blood that migrated selectively into the intima   According to Wells (74), and others, the lipid deposits in the intima may be temporary and do not lead to atherosclerosis   McMeans and Klotz (43) observed a disappearance of lipid deposits in the aorta of rabbits with experimental atherosclerosis when the feeding of cholesterol was discontinued after the aortic lesions had developed   In animals and apparently in man (Wells) lipid deposits may cause local fibrous thickenings of the intima

If we recognize that the lipids of the blood are transported in the form of small particles and that the normal intima and the simple lipid deposits of atherosclerosis have a lipid content corresponding in composition to the lipids of the blood, much of the reason for this controversy disappears   Lipids may be considered to be in particulate dispersion in the blood and interspaces of the intima tissues   Chemical analysis demonstrates this lipid material in the intima although no lipid is found by morphologic examination   An increase in the particle size of the lipids brings them into the range where phagocytosis occurs   This may be considered to be the initial stage of atherosclerosis

TISSUE REACTIONS CAUSED BY LIPIDS   The chemical analyses which demonstrated a close agreement in composition of the lipids of the blood, the normal intima, and the fatty plaques in atherosclerosis of the aorta indicate that a nonspecific infiltration of the intima by the plasma lipids occurs   Each lipid has a specific effect when deposited in tissues   The effects which the individual lipids, free and ester cholesterol, phosphatides, galactosides, fats, and their hydrolysis products, notably the fatty acids, cause in tissues must receive consideration in evaluating the significance of the fatty deposits in the subsequent changes of the intima   Cholesterol and cholesteryl oleate (28) deposited in the tissues stimulate a foreign body reaction, cerebrosides cause a pseudoxanthoma cell response, and lecithin, a polyblastic tissue reaction   With mixtures of the lipids, a differentiation on the basis of tissue reactions is impossible   This loss of characteristic tissue reactions with lipid mixtures led Kimmelstiel and Laas (33) to believe that the chemical character of lipid alone was not responsible for the tissue reactions   The fibroblastic tissue reaction with giant cells, they stated, was the response toward an insoluble substance   This reaction did not occur when cholesterol was mixed with lecithin and was highly dispersed   The types of xanthoma cell produced experimentally by such a mixture, they stated, depended upon the colloidal dispersion of the lipid and not on its chemical character

The effect of lipid mixtures on arterial tissues was investigated in dogs by Christianson (13)   The technical difficulties encountered in these experiments were considerable, and the lipid mixtures injected lodged usually in the media and not in the intima   The lipids, however, produced fibrous scars depending upon their composition   Those composed of glycerides and fatty acids caused marked inflammatory reactions and finally a scar, those composed of glycerides mixed with calcium soaps caused a chronic inflammation which also resulted in

a scar   Fibrous plaques formed in the intima over these lesions   Hagertv (23) reported that oleic acid introduced into the tissues of rabbits and dogs caused a severe necrosis and a marked growth of scar tissues   According to Hirsch (27) some of the factors involved in reactions of tissues about glycerides are the acidity developed in the surrounding tissues by their hydrolysis, the physical state of the fatty acid, whether solid or liquid, the solubility of the soaps formed and their chemical structure   Many of the fatty acids in tissues are non specific irritants, stimulating mainly fibroblastic, monocytic, and epithelioid cells   Soaps of these fatty acids, relatively insoluble in the tissues   stimulated foreign body granulation tissues

There is also much evidence in the many reports on the various forms of lipid pneumonias (53) and on the lesions of traumatic fat necrosis (26) that lipids cause marked inflammatory reactions and growths of fibrous scars in human tissues   Some of these tissue reactions develop rapidly, others evolve slowly

According to the evidence presented, lipids deposited in tissues stimulate in flammatory reactions which eventually are reduced to fibrous plaques   The different lipids in these deposits contribute variably to the sum total of the tissue reactions and among them, the fatty acids seem to have the greatest irritative properties   The factors concerned with the stabilization of the lipid particles in the tissues, also are important

THE RELATION OF THE LIPIDS TO CALCIFICATION   The absence of observed abnormalities in the calcium content of the blood suggests that local tissue factors are responsible for the calcification in atherosclerosis of the vessels   The frequent occurrence of calcification with atherosclerosis and with certain forms of fatty changes led to the opinion that calcium deposition in some way was connected with the presence of lipids   This hypothesis was emphasized by Klotz (34, 35) who claimed to have observed the presence of calcium salts of fatty acids in degenerating tissues, and to have observed infiltration of calcium into fats or fatty acids imbedded in tissues of the body   These conclusions were based on microscopic observations alone, and though often quoted, this theory has not been confirmed   Aschoff (4) suggested that the formation of calcium soaps preceding calcification occurs through splitting of cholesterol esters, whereby the fatty acids liberated combine with calcium ions to form calcium soaps and the fatty acid is replaced gradually by phosphate and carbonate ions   If Aschoff's theory were correct, the ratio of cholesterol esters to cholesterol should decrease with gradual evolution of the atherosclerotic lesions   In an investigation of this Schönheimer (61) observed no change in the ratio and concluded therefore that Aschoff's theory was incorrect   Subsequently how ever, Weinhouse and Hirsch (71) and Meeker and Jobling (45) observed a lowered cholesterol-ester to cholesterol ratio in calcified atherosclerotic lesions

As Hirsch (26, 27) has pointed out, chemical combination between positive ions in an aqueous phase and negative ions in the lipid phase may account for the occurrence of chemical reactions between the two systems   In support of this view are the observations of Langmuir and Schaefer (38), who found that fatty acids in the form of monomolecular films floating on solutions of calcium

and barium salts, were converted to the corresponding soaps to varying extents, depending upon the pH of the aqueous medium   Similar results were reported by Hartsuch (24), who found that magnesium ions were lost from a dilute aqueous solution of magnesium phosphate when shaken with oleic acid   The transfer of magnesium ions was appreciable at a pH of 7   There is, however, much other evidence refuting Aschoff's hypothesis, mainly obtained by Wells (74) and discussed by him at length   Briefly summarized, the evidence against the idea that calcification takes place through intermediate reaction with lipids is as follows 1, calcium soaps have never been isolated from calcified aortas or from any other calcified pathological process   This fact has been demonstrated by Baldauf (6), Wells (73), and in unpublished experiments by Weinhouse and Hirsch, 2, even if calcium soaps were present in the body, calcification may not occur, for Wells (75) has introduced calcium soaps into living tissues and when removed they had not been converted into inorganic salts, and 3, the composition of the calcified portion of atherosclerotic aortas closely resembles that of bone   Accordingly, the mechanism of calcification with atherosclerosis is similar to that with bone formation   The analyses, reviewed by Wells (74), agree that the calcium in the lesions of atherosclerosis is mainly in the form of calcium phosphate, and small, variable amounts of carbonate and magnesium salts   The analogy to bone formation is emphasized by the development of bone tissues in old lesions   The similarity of calcified intimal lesions to true bone was demonstrated by Mr Raymond Pepinsky of the Physics Department of the University of Chicago, who at our request investigated the x-ray diffraction patterns of several calcified atheromatous lesions   These patterns were identical with those given by minerals of the apatite group and were identical with the diffraction patterns of bone observed by Taylor and Sheard (64)   According to these authors the inorganic portion of bone is composed of the apatite, $3Ca_3(PO_4)_2$ $CaCO_3$

Chemical and physical evidence favor the opinion that calcification with atherosclerosis is a secondary process similar to the calcification in other tissues in the body where necrosis has occurred

THE COMPOSITION OF THE BLOOD AND TISSUE LIPIDS IN EXPERIMENTAL ATHEROSCLEROSIS   The lesions of the aorta and other tissues in the experimental form of atherosclerosis produced in animals by diets containing added amounts of cholesterol have been described fully by Anitschkow (3), Duff (18) and Leary (39, 40)   The similarity between the human and experimental forms of atherosclerosis has been emphasized regardless of certain obvious differences   Atherosclerosis develops in rabbits only after a significant hypercholesteremia has been present   Hypercholesteremia has not been observed usually in human atherosclerosis   The deposition of the lipids in the wall of the aorta and arteries in the experimental form of atherosclerosis is part of a systemic lipid infiltration of the tissues

Blood   A daily intake of cholesterol of less than one per cent of the diet in rabbits produces a significant hypercholesteremia in a week or less, and in several months the blood cholesterol may reach high levels   Values over 2500 mgm

per 100 cc of serum have been observed frequently   The first observations of
hypercholesteremia were recorded by Wacker and Hueck (69), who reported a
twenty fold increase in both free and total cholesterol of the blood after several
months of the cholesterol diet   These observations were confirmed by Versé
(67), who noted that the cholesterol levels of the blood increased more rapidly
and with smaller daily feedings when the cholesterol was given dissolved in oil
The most extensive studies of the blood lipid changes in experimental athero-
sclerosis were reported by Page and Bernhard (49) and Weinhouse and Hirsch
(71)   These studies established that in addition to the cholesterol, there is a
marked increase in the other lipids, notably the phospholipids and the glycerides
A comparison of the results of both analyses is in table 4

At the height of lipemia there was an average increase of twenty five to thirty-
fold in the free and total cholesterol. Contrary to earlier reports there was no
significant increase in the proportion of cholesterol esters   Despite the marked
increase in total cholesterol the average ratio of free to total cholesterol, 0.28

TABLE 4

Blood lipid levels at height of lipemia

INVESTIGATOR‡	FREE CHO-LESTEROL	TOTAL	ESTERS	PHOSPHO-LIPID	GLYCERIDES	TOTAL LIPIDS
Page and Bernhard*	356	1435	1823	520	270	2902
Weinhouse and Hirsch†	452	1652	2010	706	718	3660

* Cholesterol administered as a solution in oil 0.2 gram per day   Determinations made
on heparinized plasma

† Cholesterol administered without added fat 1 0 gram per day   Determinations made
on serum

‡ *Dauber and Katz (14) recently reported similar results with experimental atherosclero-
sis in chickens*

at the height of lipemia, was the same as in the normal rabbits   Phospholipids
increased to seven times the normal value   whereas glycerides increased to five
times normal   The total lipids were on the average, eleven times the normal
value   The increase in the blood lipids began in a few days, reached a maximum
usually between fifty and one-hundred days on the cholesterol diet, and there-
after the amount declined   In animals surviving more than one hundred and
fifty days under the conditions of the experiment, the blood lipids approached
normal values   This decrease does not indicate development of a tolerance for
cholesterol in these animals, because at this stage they lost weight rapidly and
exhibited all the signs of starvation as though the normal absorption of food
across the intestinal wall was interrupted   The tissues of the stomach and
bowel of these rabbits had marked intracellular infiltrations of lipids

The effect of added fats to the diet in facilitating the absorption of cholesterol
and in increasing the lipemia is illustrated also in table 4   At the height of
lipemia, both studies exhibit about the same lipid levels, although Page and Bern-
hard fed their rabbits only one fifth the quantity of cholesterol   They administ-

tered it dissolved in oil   These analyses of the lipids of the blood in experimental atherosclerosis demonstrated clearly that not only is the cholesterol content increased to high levels, but also all of the other lipids   In studies of the physical state of the blood lipids in experimental hyperlipemia in rabbits, Remesow (56) suggested that the increase in the lipids other than cholesterol may be due to a destruction or binding of the serum "lipases" by the excess cholesterol, thus causing the accumulation of lipids   This opinion is based on the inhibition of lipases by alcohols in general   However, the effect of excess cholesterol in causing increases in other blood lipids may not be due to any chemical effect of this sterol, but to its effect on the physical state of the blood lipids   The increase in hydrophobic components of the blood lipids, such as cholesterol and its esters, presumably could decrease the stability of the lipid complex, leading to a decrease in the dispersion of the colloid   Agglomeration into larger particles would follow and a marked decrease in the surface area exposed to enzyme activity would result

Tissues   Weinhouse and Hirsch (72) found, in rabbits fed cholesterol, marked increases in the lipids of the viscera as well as of the aorta   The greatest changes were in the aorta, liver, and suprarenal glands   The lungs, kidneys, and spleen occasionally had lipid infiltration and correspondingly high cholesterol or cholesterol ester values, but usually there were only minimal changes in lipid content   The atherosclerotic aortas had a marked increase in free and total cholesterol as well as a significant though variable increase in the phospholipids and glycerides   The total lipids were, in general, increased two or three times above the level of those in the normal aortas   The livers of all rabbits fed cholesterol contained quantities of lipids substantially above the levels in control rabbits, the increase being greatest in the cholesterol ester fractions and least in the glycerides   The cholesterol content of the suprarenal glands was markedly increased   The free cholesterol was increased from two or three times above the content of normal tissues, and the cholesterol esters, which in the control analyses averaged 14 per cent, were doubled in the cholesterol-fed rabbits These analyses of the suprarenal gland agree with those of Kay and Whitehead (31)

The significance of the hyperlipemia in experimental atherosclerosis   The relationship between hypercholesterolemia and atherosclerosis in the rabbit seems to be definite   The lesions of experimental atherosclerosis in rabbits, which closely resemble those of the human form, have not been produced in any other way Despite variations in diet and superimposed types of mechanical and chemical injury, an excess of cholesterol in the blood appears to be necessary in the formation of the lipid plaques of the aorta and arteries   The evidence implicating cholesterol as a primary agent in the development of atherosclerosis has been presented forcefully by Leary (39)   According to his views, the differences between human and experimental atherosclerosis may be attributed to differences in reaction of youthful and aged tissues to the presence of cholesterol and not to any fundamental difference in etiology

In the experiments of Weinhouse and Hirsch (72), though hypercholesterolemia always preceded deposition of cholesterol, there was no close relationship between

the height or duration of the hypercholesteremia and the severity of the athero sclerosis For example, of two rabbits fed the same daily amounts of choles terol for the same period of time, the serum cholesterol of one reached a maxi mum of 2200 mgm per cent, the other, 1500 mgm per cent The severity of atherosclerosis as determined by gross examination, and the amount of lipids deposited, were about the same In two other rabbits with the same degree of hypercholesterolemia, one had severe, and the other had only a mild atherosclero sis These results logically suggest that tissue factors play an important part in the development of experimental atherosclerosis

TISSUE FACTORS IN ATHEROSCLEROSIS The results of these chemical analyses indicate that tissue factors are important in the development of atherosclerosis in man and experimentally in rabbits Weinhouse and Hirsch (72) did not find a close correlation between the height and duration of lipemia and the severity of the experimental atherosclerosis These individual variations were found also in different viscera of the same rabbit, some visceral tissues being markedly infiltrated by lipids and others relatively unchanged

The importance of tissue factors in the development of experimental athero sclerosis is indicated also by studies on the effects of iodine and thyroid hor mone. According to Page and Bernhard (49), and Turner and Bidwell (65) the addition of iodine or thyroid extract to the diet prevents or inhibits the develop ment of atherosclerosis in rabbits fed cholesterol, although these substances did not prevent or delay the hyperlipemia

Since the atherosclerotic lesions are not distributed diffusely through the vessel wall but are sharply localized, certain places in the arteries are more susceptible to lipid infiltration than others The lesions tend to be more numer ous in tissues subject to unusual mechanical strain or at sites with variations in tissue structure as, for example, along the posterior wall of the descending aorta at the ostiums of arterial branches and at the bifurcation of the iliac branches This suggests that conditions increasing the mechanical stress, such as hyper tension, contribute to the development of atherosclerosis As the cause and effect relationships are not clear, probably a large variety of conditions are con cerned with this localization of the lesions If hypercholesteremia alone played an important rôle in human atherosclerosis there should be a closer relationship between the disease and the blood lipids than has been demonstrated Although hyperlipemia may favor the development of atherosclerosis factors in the tissues are concerned with the actual lipid deposition

MECHANISM OF LIPID DEPOSITION The significance of the lipid deposits in the intima with atherosclerosis is disputed Opinions differ as to whether the lipids at first are intracellular and with destruction of the cells become extra cellular or at first are extracellular and secondly become intracellular

Chemical analyses by Weinhouse and Hirsch (71), revealing a close agreement in the composition of the blood plasma lipids and that of the normal intima tissue, suggest that the intercellular spaces of the intima are filled with blood plasma Accordingly, reactions which take place in the blood would be ex pected to occur also in the intima The blood lipids of the plasma exist as colloi dal complexes, ordinarily too small to be seen microscopically In hyperlipemia,

however, the lipid complex agglomerates into larger particles, which are visible under the microscope and which give to such serums an opalescence   It is reasonable to conclude, therefore, that the phagocytic reactions observed in the intima occur as a response to the presence of coarsely dispersed lipids in varying stages of agglomeration   The primary cause of the precipitation of lipid in cholesterol-fed rabbits is probably the hyperlipemia, in human atherosclerosis it is mainly a tissue factor increased under conditions of hyperlipemia   Regardless of the cause of the precipitation of lipids, however, all the subsequent chemical processes and tissue reactions may be regarded as sequential to the initial separation of lipid in grossly dispersed particulate form

SUMMARY

Chemical, physical and morphologic changes occur in arteries with age   These changes seem to be independent of atherosclerosis which is primarily a disease of the intima   The simple fatty deposits with atherosclerosis have the same lipid composition as the blood plasma and the normal intima   The identical composition of these tissue lipids strongly supports the view that the lipid deposits in atherosclerosis result from a non-selective deposition of the plasma lipids   Although cholesterolemia may favor the development of atherosclerosis, the lesions of this vascular disorder develop in adult life without an appreciable elevation of the blood cholesterol or obvious abnormality of the blood lipids   This suggests that factors in the tissues of the blood vessels leading to the deposition of lipids in the intima are important in the causation of the disease   The tissue factors concerned with the *localization of the lipid deposits in the intima* are not known   However, after the lipids are deposited and remain in the tissues in coarse particulate form, the subsequent stages of atherosclerosis follow   The evolution of the lesions from the simple fatty deposits to calcified atheromatous ulcers occurs because of several processes, among which are phagocytosis, physical or chemical effects on the tissues by the lipids or their decomposition products, disturbances in nutrition of the tissues, admixtures of blood, and reactions of the tissues against the lipids deposited   The calcification which occurs late in the evolution of the lesions probably is the same as that which develops in any necrotic or devitalized tissue of the body   The evidence presented in this review favors the conclusion that atherosclerosis develops from disturbances in the metabolism of lipids infiltrated into the tissues of the intima   The elucidation of the nature of the disturbances, however, depends upon further fundamental studies on the interactions and interconversions of the lipids in the blood and tissues

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THE NATURE OF THE FORCES BETWEEN ANTIGEN AND ANTIBODY AND OF THE PRECIPITATION REACTION

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In one of his lectures on immunochemistry at the University of California in the summer of 1904 Svante Arrhenius said (1) that Ehrlich and other investigators, because of incomplete knowledge of the phenomenon of chemical equilibrium had been led to invent artificial hypotheses in order to explain their observations in the field of immunology   Since that time, and especially during the last few years, workers in this field have made greater and greater use of the concepts and methods of physical chemistry, and in consequence many previously puzzling observations have been reasonably interpreted.

Another branch of chemistry which is of importance to immunology is modern structural chemistry, which deals with the detailed structure of molecules and with the nature of interatomic and intermolecular interactions (2)   Our present knowledge of this subject, in large part won during the past dozen years, is now so firmly founded and so extensive that it can be confidently used as the basis for a more penetrating interpretation of immunological observations than would be provided by the observations alone

In this paper we present, after a brief historical introduction, a discussion of the nature of the specific forces between antigen and antibody and of the precipitation reaction from the point of view of modern chemistry   Only the simpler aspects of the phenomena are discussed, such complicating factors as the rôles of complement, lipids, etc , in the reactions are disregarded in our discussion

The history of the precipitation reaction began in 1897, when Rudolf Kraus (3) reported the results of his work with anticholera and antityphoid sera.   His observations were soon verified and extended by Nicolle Tchistovich, Bordet, Myers and other workers, who prepared precipitating antisera against a great number of antigens of varied nature.   We shall not review this early work here nor the later studies of the methods of preparing antisera and carrying out the precipitation reaction, since these topics and others dealing with special phases of the reaction have been very well covered in earlier reviews (4, 5, 6)

Two most important advances in the attack on the problem of the nature of immunological reactions were the discovery that the specific precipitate contains both antigen and antibody (7) and the discovery that antibodies which give antisera their characteristic properties, are proteins.   The verification of these facts was provided by the work of many investigators over a score of years   This work, which is summarized in Marrack's monograph (6, chap II), culminated in the preparation of purified antibody by Felton and Bailey (8), Heidelberger and collaborators (9), and others and the determination of its properties including amino-acid composition and molecular weight, which show that it is very closely related to normal serum globulin (6, chap II)

The work of Landsteiner (10) and other investigators on artificial conjugated antigens provided a great body of qualitative information on the specificity of antibodies, which, together with the experimental results for natural antigens, led to the independent proposal by Breinl and Haurowitz (11), Alexander (12), and Mudd (13) in 1930–32 of the theory of structural complementariness of antigen and specific antibody   The framework theory of precipitation was then developed by Marrack (6) and Heidelberger (14)   These and other theories are discussed in some detail in the following sections of this paper

A new period in the study of the precipitation reaction was initiated by the careful quantitative studies of Heidelberger and his collaborators (15) who determined the amounts of antibody and antigen in precipitates, and the similar work of Haurowitz (16) and others   Very recently, in order to test certain aspects of his detailed theory of the structure of antibodies (17), Pauling and his collaborators have carried out many quantitative experiments on the precipitation of antisera by polyhaptenic simple substances (18, 19, 20), a phenomenon first observed by Landsteiner and Van der Scheer (21)

THE NATURE OF THE SPECIFIC FORCES BETWEEN ANTIGEN MOLECULES AND ANTIBODY MOLECULES   The detailed information which has been gathered in recent years regarding the nature of the chemical bonds which hold atoms together into stable molecules has been summarized in monographs (2, 22)   Instead of interacting strongly with one another, with interaction energy of 20 kilocalories per mole or more, to produce a chemical bond, two atoms may interact more weakly   The nature of these weak interactions is now well understood, and a brief discussion of it is given in the following paragraphs   The properties of antigen-antibody systems, especially the reversibility of complex formation, are such as to indicate that the antigen-antibody attraction is due to these weaker interactions and not to the formation of ordinary chemical bonds

The weak interactions between two molecules may be classified as electronic van der Waals attraction, Coulomb attraction, attraction of electric dipoles or multipoles, hydrogen-bond formation, etc   The forces increase rapidly in magnitude as the molecules approach one another more and more closely, and the attraction between the molecules reaches its maximum when the molecules are as close together as they can come   The molecular property which determines the distance of closest approach of two molecules is the electronic spatial extension of the atoms in the molecules   It is possible to assign to each atom a *van der Waals radius*, which describes its effective size with respect to intermolecular interactions   These radii vary in value from 1 2 Å for hydrogen through 1 4–1 6 Å for light atoms (fluorine, oxygen, nitrogen, carbon) to 1 8–2 2 Å for heavy atoms (chlorine, sulfur, bromine, iodine, etc)   The shape of a molecule can be predicted by locating the atoms within the molecule with use of bond distances and bond angles and then circumscribing about each atom a spherical surface corresponding to its van der Waals radius   This shape determines the ways in which the molecule can be packed together with other molecules (2, sec 24)

The most general force of intermolecular attraction, which operates between

every pair of molecules, is *electronic van der Waals attraction* This type of electronic interaction between molecules was first recognized by London (23) A molecule (of methane, for example) which has no permanent average elec tric dipole moment may have an instantaneous electric dipole moment, as the center of charge of the electrons in their rapid motion in the molecule, swings to one side or the other of the center of charge of the nuclei This instantaneous dipole moment produces an instantaneous electric field, by which any other molecule in the neighborhood would be polarized, the electrons of the second molecule would move relative to its nuclei in such a way as to give rise to a force of attraction toward the first molecule.

This electronic van der Waals attraction operates between every atom in a molecule and every atom in other molecules in the near neighborhood The force increases very rapidly with decreasing interatomic distance, being inversely proportional to the seventh power of the interatomic distance Hence the electronic van der Waals attraction between two molecules in contact is due practically entirely to interactions of pairs of atoms (in the two molecules) which are themselves in contact, and the magnitude of the attraction is determined by the number of pairs of atoms which can be brought into contact In conse- quence, two molecules which can bring large portions of their surfaces into close- fitting juxtaposition will in general show much stronger mutual attraction than two molecules with less extensive complementariness of surface topography

Other types of molecular interactions result from the possession of a perma nent electric charge, electric dipole moment, or electric moment of higher order by one or both of the interacting molecules The effects of these charges and moments have been classified in various ways, as ion ion forces, dipole-dipole forces, forces of electronic polarization of one molecule in the dipole field of another, etc. All electrostatic interactions are very much smaller in water than in a medium of low dielectric constant, and it can be shown by calculation, mak ing use of known values of the effective dielectric constant of water for charges a given distance apart (24), that in general these electric forces are of minor importance, except when an isolated or essentially isolated electric charge is involved. The electrostatic attraction of a positive group such as a substituted ammonium ion and a negative group such as a carboxyl ion becomes significantly strong, with bond energy 5 kilocalories per mole or more, if the structure of the molecules containing the groups is such that they can come into juxtaposition

A type of intermolecular attractive force which ranks in importance with the electronic van der Waals attraction and the attraction of oppositely charged groups is that associated with the structural feature called the *hydrogen bond* The importance and generality of occurrence of the hydrogen bond were first pointed out in 1920 by Latimer and Rodebush (25) and summaries of the proper ties of the bond are given in the monographs quoted above A hydrogen bond results from the attraction of a hydrogen atom attached to one electronegative atom for an unshared electron pair of another electronegative atom The strength of a hydrogen bond depends on the electronegativity of the two atoms which are bonded together by hydrogen, fluorine, oxygen, and nitrogen the

most electronegative of all atoms, are the atoms which form the strongest hydrogen bonds   The energy of a hydrogen bond between two of these atoms is of the order of magnitude of 5 kcal per mole   This is so large as to have a very important effect on the intermolecular interactions of molecules capable of forming hydrogen bonds and on the properties of the substances consisting of these molecules

In synthesizing our knowledge of intermolecular forces and of immunological phenomena into a definite picture of the antigen-antibody bond the immunological property of greatest significance is the specificity of the combining power of antibody for the immunizing antigen

The forces of van der Waals attraction, hydrogen-bond formation, and interaction of electrically charged groups are in themselves not specific, each atom of a molecule attracts every other atom of another molecule by van der Waals attraction, each hydrogen atom attached to an electronegative atom attracts every other electronegative atom with an unshared electron pair which comes near it, and each electrically charged group attracts every other oppositely charged group in its neighborhood   The van der Waals repulsive forces which determine the van der Waals radii of atoms also are not specific, each atom in a molecule repels every other atom of another molecule, holding it at a distance corresponding to the sum of the pertinent van der Waals radii   We see, however, that specificity can arise in the interaction of large molecules as a result of the shapes of the molecules   Two large molecules may have such spatial configurations that the surface of one cannot be brought into contact with the surface of the other except at a few isolated points   In such a case the total electronic van der Waals attraction between the two molecules would be small, because only the pairs of atoms near these few isolated points of contact would contribute appreciably to this interaction, and, moreover, the distribution of hydrogen-bond forming groups and of positively and negatively charged groups of two molecules might be such that only a small fraction of these groups could be brought into effective interaction with one another for any position and orientation of one molecule with respect to the other, the energy of attraction of these two molecules would then be small   If, on the other hand, the two molecules possessed such mutually complementary configurations that the surface of one conformed closely to the surface of the other, if, moreover, the electrically charged groups of one molecule and those of the other were so located that oppositely charged groups were brought close together as the molecules came into conformation with one another, and if the hydrogen-bond forming groups were also so placed as to form the maximum number of hydrogen bonds, the total energy of interaction would be very great, and the two molecules would attract one another very strongly   We see that this strong attraction might be highly specific in the case of large molecules which could bring large areas of their surfaces into close contact   A molecule would hence show strong attraction for another molecule which possessed complete complementariness in surface configuration and distribution of active electrically charged and hydrogen-bond forming groups, somewhat weaker attraction for those molecules

with approximate but not complete complementariness to it, and only very weak attraction for all other molecules

This specificity through complementariness of structure of the two inter acting molecules would be more or less complete, depending on the greater or smaller surface area of the two molecules involved in the interaction   It may be emphasized that this explanation of specificity as due to a complementariness in structure which permits non-specific intermolecular forces to come into fuller operation than would be possible for non-complementary structures is the only explanation which the present knowledge of molecular structure and inter molecular forces provides

This theory of structural complementariness of antigen and antibody was first suggested in less detailed form than above, by Breinl and Haurowitz (11), Alexander (12), and Mudd (13)   A detailed discussion of the structure of antibodies and of a postulated method of their formation has been presented by Pauling (17), who has also reviewed the evidence supporting the theory of complementariness

It was suggested by Breinl and Haurowitz and by Mudd that the effect of an antigen in determining the structure of an antibody might involve the ordering of the amino-acid residues in the polypeptide chains in a way different from that in the normal globulin   Rothen and Landsteiner (26) then pointed out that the possibility of different ways of folding the same polypeptide chain is worth considering, and this postulate was amplified by Pauling (17), who assumed that all antibody molecules contain the same polypeptide chains as normal globulin, and differ from normal globulin only in the configuration of the chains. This assumption was made because it permits the formulation of a simple proposed mechanism of manufacture of specific antibodies   An antibody mole cule capable of existing in any one of a great number of configurations with nearly the same energy is synthesized, except for the final folding step, in the same way as normal globulin   If no foreign substance is present, the chain then folds into a stable configuration, characteristic of normal globulin, but if an antigen molecule is present, the chain folds into a configuration stable in the presence of the antigen, that is into a configuration complementary to that of a portion of the surface of the antigen molecule   This explanation of the ability of an animal to form antibodies with considerable specificity for an apparently unlimited number of different antigens (27), as shown especially by the work of Landsteiner (10), is compatible with the principles of structural chemistry and thermodynamics as well as with the immunological evidence

To illustrate the way in which the complementariness theory accounts for many reported observations we shall mention only one point, taken from the great body of results on azoproteins obtained by Landsteiner   He observed a pronounced cross reaction between an azoprotein made from m aminobenzoic acid and an antiserum to an azoprotein made from 4-chloro-3-aminobenzoic acid and a different protein but no reaction with the haptenic groups reversed The explanation of this is that the antibody to the 3 azo-4-chlorobenzoic acid group conforms closely to this haptenic group, allowing either this group or the

3-azobenzoic acid group, which differs in the replacement of the chlorine atom by a smaller atom, hydrogen, to fit into the complementary cavity in the antibody, but the 3-azo-4-chlorobenzoic acid group cannot fit into a cavity designed for the smaller haptenic group, and so the reverse cross reaction does not occur In a quantitative extension of Landsteiner's work on hapten inhibition of precipitation reactions of simple polyhaptenic substances (10), Pauling and collaborators (28) have recently reported a great deal of evidence in support of the complementariness theory   They interpreted their results on the inhibition of the precipitation reaction between dyes containing p-azophenylarsonic acid groups and antisera to hapten-homologous azoproteins to obtain numerical values of the strength of the bonds formed by these antibodies with over twenty-five different haptens   The observed correlation between the bond strengths and the structure of the haptens is that which would be expected from the complementariness theory

This theory is not greatly different from some earlier proposals, such as Ehrlich's lock-and-key analogy (29), but it differs greatly from others   For example, Buchner (30) considered that antigen molecules are split up and incorporated into the antibody molecules, thus imparting specificity to them This theory or a closely related theory has been supported by many people, including Burnet (31), who proposed a mechanism for the manufacture of antibodies in the image of the antigens the antigens act as templates for the manufacture by the body of specific enzymes, which then serve as the molds for the production of antibodies similar to the original antigens   Until recently there has been no suggestion as to why antibodies similar in structure to an antigen should combine specifically with it   Recently, however, Jordan (32) has stated that a strong attraction would occur between such identical or nearly identical molecules because of the quantum-mechanical resonance phenomenon, this has been denied by Pauling and Delbrück (33), who pointed out that the resonance energy would be so small as to be ineffective   Chemical evidence against the identity of antibodies and specific antigens has been presented by many authors, of whom the most recent are Haurowitz, Vardar, and Schwerin (34)

Forty years ago there was under way a keen controversy between Ehrlich and Bordet, and their respective supporters, as to whether the bonds between antibodies and antigens are chemical bonds or are physical forces of the sort producing surface phenomena such as adsorption   The modern point of view resolves this argument, but not in favor of either side, in fact, as in recent years an understanding has been obtained of the forces responsible for surface phenomena it has been found that these forces are the same as those which are operative in chemical reactions, so that the old distinction between chemical and physical forces has lost most of its meaning

THE NATURE OF THE PRECIPITATE   Under suitable conditions (salt concentration, antibody-antigen ratio, etc) the first stage of combination of antibody and antigen, which may make itself evident in change in toxicity or other properties of the antigen, is followed by precipitation   There has been much discussion as to whether or not this second stage is specific, like the first stage,

or is non-specific   Direct experimental evidence on this point, while not conclusive, favors the view that the reaction is specific. The most pertinent observations are those on the agglutination of mixed cellular antigens by mixed antisera, Topley and collaborators (35) noted the formation of separate clumps by the different cells, whereas Abramson (36) observed mixed clumping   Hooker and Boyd (37) found that mixed human and chicken erythrocytes gave separate clumps under some conditions and mixed clumps under other conditions   The fact that separate clumping is observed at all strongly favors the concept of a specific second stage, since mixed clumping might result from mechanical intertwining of specific clumps, whereas separate clumping would hardly be expected to result from non specific interaction Heidelberger has pointed out that in those cases where mixed clumping takes place the cells used were either very large or of greatly different sizes (38)

A reasonable theory of agglutination and precipitation, the framework theory (lattice theory[1]), was proposed in 1934 by Marrack (6), and has received strong support from the theoretical considerations and experiments of Heidelberger (9, 14, 15) and Pauling (18, 19, 20, 28) and their collaborators

It is clear that, after we have accepted a mechanism for the specific attachment of antibody molecules to a cellular antigen, the simplest possible explanation of the agglutination of the cells is that it results from the same mechanism, if an antibody molecule had the power of specific attachment to two cells, it could form specific bonds with the two cells and thus hold them together, and the repetition of this process would lead to the formation of larger and larger clumps Specific precipitation of antibodies and molecular antigens would result from the same mechanism if both antibody molecules and antigen molecules were multivalent (capable of forming two or more antigen-antibody bonds), larger and larger complexes A—B, A—B—A, A—B—A—B, etc., would form until the aggregates became macroscopic in size   The evidence supporting the framework theory has been reviewed by Marrack (6), Heidelberger (14), and Pauling (17), some of it, including that provided by recent work, is presented in the following section in connection with a discussion of the valence of antibody molecules

The first of the theories of non-specific precipitation is the theory of neutralization of electrical charges   This theory was supported by many early investigators, who were attracted by the analogy with the well known phenomenon of the mutual precipitation of oppositely charged colloids   Teague and Field (39) investigated the charges of agglutinins and bacteria and concluded that the former are positively and the latter negatively charged, it is now known, however, as the result of the application of improved experimental methods, that under ordinary conditions (normal hydrogen ion and salt concentrations) antibody molecules and most antigens are negatively charged, and the theory of neutralization has in consequence been abandoned

(The failure of precipitation or agglutination to occur in antigen antibody

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[1] We have adopted the name "framework theory instead of 'lattice theory' because of our belief that the framework of antibody antigen precipitates does not usually have the regularity of structure which would be indicated by use of the latter expression

systems with low salt concentration is, indeed, attributed to the electrostatic repulsion of the negatively-charged complexes in solution, which prevents the formation of large aggregates, agglutination or precipitation may occur in the presence of salt, the cations of which neutralize the negative charges of the complexes)

Another theory of non-specific precipitation which was proposed soon after the discovery of the precipitation reaction is that the reaction results from the formation of a hydrophobic colloid, which precipitates in the presence of electrolytes   This theory has been revived recently by Eagle (40) and by Hooker and Boyd (37)   Eagle's suggestion that the polar groups of the antigen which are assumed to be responsible for its solubility are masked by a layer of antibody molecules, which themselves turn their polar groups inward and present only non-polar groups toward the solvent, has been discussed by Marrack (6), who has marshalled some arguments against it   An important argument is that particles can be agglutinated by an amount of antibody very much smaller than the amount required to coat their surface, the most recently reported experiments of this sort (41) indicate that azoerythrocytes can be agglutinated by less than 0 02 per cent as much antibody as would cover their surface with a layer 3 5 Å thick

Hooker and Boyd (37) have presented several arguments in support of the thesis that "   particles grow to visible size by the indiscriminate and non-specific accretion of other related or unrelated, small or large, aggregates whose primary nuclei are molecules or particles of antigen coated with antibody-globulin "   As additional evidence for this concept and against the framework theory Boyd and Hooker (42) reported their failure to inhibit the agglutination of erythrocytes by use of an excess of hemagglutinin   In our opinion the fact that inhibition of agglutination of particles (43) as well as of precipitation of molecular antigens (44) by excess antibody has been observed gives strong support to the framework theory   The failure of inhibition to occur under ordinary circumstances may be due to the difficulty in saturating the multivalent antigens, especially cellular antigens with thousands of combining groups, as is indicated by the theories of Hershey (45) and Pauling (17)   In particular, the experiments of Heidelberger and Kabat (43) on the agglutination by untreated pneumococci of pneumococci coated with antibody and then thoroughly washed are most easily explained by the framework theory   Hooker and Boyd (46, 47) have recently proposed the theory that precipitation of antibody by polyhaptenic dyes may result from the action of the dye molecules in pulling the antibody molecules to which they are bonded so tightly together as to prevent the solvent from reaching the polar groups   This theory seems to be incompatible with our observation (18) that in general the dyes of smaller molecular size, which according to Boyd's theory should pull the molecules more closely together, in fact precipitate less completely than those of larger molecular size

COMPOSITION OF ANTIBODY-ANTIGEN PRECIPITATES AND VALENCE OF ANTIBODY

An essential requirement for agglutination or precipitation according to the framework theory is that both antigen and antibody be multivalent   The

experimental observations which indicate multivalence of antigens and of agglutinins and precipitins have been summarized by Marrack (6), Heidelberger (14), and Pauling (17)

The most straightforward evidence for the necessary multivalence of antigen is given by experiments on the reactions of antibodies with simple substances of known structure. Subsequent to Landsteiner's discovery (10) that simple haptens inhibit the precipitation and agglutination reactions by forming soluble complexes with antibody it was found by Landsteiner and Van der Scheer (21) that simple substances containing two or more haptenic groups form precipitates with hapten homologous antibodies   We have shown that of the twenty seven simple substances containing phenylarsonic acid groups which were tested with antisera made by injecting rabbits with azoprotein made from p-arsanilic acid each of those (twenty in number) which contained two or more of the haptenic groups gave the precipitation reaction, whereas none of the monohaptenic substances formed precipitates   These facts support the framework theory strongly

(The failure to obtain precipitates with some polyhaptenic substances reported by Hooker and Boyd (46) and Boyd (47) may have been due to their failure to work under conditions favorable to precipitation   We have obtained precipitates with some of the same substances and have observed that substances which give precipitates with strong antisera may fail to do so with weak antisera)

Experiments which have been reported on the number of haptenic groups per azoprotein molecule necessary for precipitation with hapten homologous antibody (48, 49) and some of our unpublished results indicate that a few groups are needed, but so far they have not been precise enough to distinguish between 1 and 2 as the minimum

Direct proof of the bivalence of diphtheria antitoxin is given by the studies of the antitoxin in presence of an excess of toxin with use of the ultracentrifuge which showed that the complexes ToxAntitox and Tox₂Antitox exist in the solution (50)

The fact that slides can be coated with alternate unimolecular layers of antigen and antibody in specific combination (51 52) indicates effective bivalence of antibody molecules as well as antigen molecules

It has long been known that in antigen antibody precipitates molecules of antibody are present in larger numbers than those of antigen, the antibody antigen molecular ratio being considerably greater than unity for nearly all systems (6, p 161, 53)   This was shown convincingly by the accurate quantitative investigations of Heidelberger and his collaborators (54)   A simple explanation of this fact which does not follow directly from the framework theory in its original form (6, 14), is given by the theory as modified by Pauling (17), who made the assumption that antibodies in general are at the most bivalent   (This assumption was made because his proposed structural theory of the process of formation of antibodies is such as to make unlikely the occurrence of antibodies of higher valence)   The maximum valence N of antigens toward homologous antibodies is assumed to be determined by the sizes and shapes of the

antigen and antibody molecules, being equal to the number of antibody molecules which, when bonded to the antigen molecule, can be packed around it If the antigen and antibody molecules were spheres of the same size, this number would be $N = 12$, for smaller antigen molecules it would be smaller, and for larger ones it would be larger

The predicted antibody-antigen molecular ratio for small antigen molecules would be $N/2$ at the equivalence zone, with the maximum valences of both antibody and antigen effective, the limiting values of the ratio for antigen excess would be 1, and for antibody excess $N - 1$ For very large antigens the expected ratio at the equivalence zone would be less than $N/2$ These predictions are in reasonably good agreement with Heidelberger's observations (54, 17), which correspond to the following values of N ovalbumin and R-salt-azobiphenylazoovalbumin (molecular weight 40,000–46,000), $N = 6$, serum albumin (m w 67,000), $N = 6$ to 8, thyroglobulin (m w 700,000), $N = 30$ to 40 Data of other investigators (55, 56, 57, 58, 59, 60, 61) correspond to similar values of N, with assumed bivalence of antigen

If the valence of the antigen were known, measurement of the antibody-antigen molecular ratio could be interpreted to give the valence of antibody molecules The only reliable experiments of this sort which have been reported so far are those of Pauling, Pressman, and Ikeda (20) with simple antigens of known structure They found that the dihaptenic antigens 2-methyl-4,6-di(p-azophenylarsonic acid)phenol and 2-methyl-4,6-di(p-azobenzene(p-azophenylarsonic acid))phenol gave with antisera homologous to the p-azophenylarsonic acid group precipitates with the same molecular ratio throughout the range of relative concentrations from antibody excess to antigen excess This is what would be expected if both antibody and dihaptenic antigen were bivalent, the predicted molecular ratio under all conditions then being 1, corresponding to the structure —A—B—A—B—A—B—A—B— for the precipitate The observed molecular ratio (average of 119 analyses) was 0 75 The same independence of molecular ratio on relative concentration was found also for the four trihaptenic and tetrahaptenic substances studied, this was interpreted as resulting from the effective bivalence of these molecules also, as the result of their small size in comparison with the antibody molecules The average molecular ratios found for the trihaptenic and tetrahaptenic substances, 0 85 and 0 83, respectively, are only slightly less than unity These results, with assumed effective bivalence of the antigens, indicate for antibody molecules the effective valence 2 3

Substantiating evidence for the multivalence of precipitating antibodies is provided by the observations which have been interpreted as resulting from the presence in antisera of antibodies with a valence of one Heidelberger and Kendall (62) demonstrated the presence in rabbit antisera of univalent antibodies, which are able to combine specifically with antigen but are not able, in the absence of multivalent antibodies, to form precipitates These univalent antibodies also occur in considerable amount in horse antisera, they seem to be produced in large amount by the first injections of ovalbumin into horses, precipitating (multivalent) antibody being formed only on repeated

injection (63, 64)   It is probable that univalent antibody confers on horse antitoxins the peculiar properties which they show, in particular the pronounced prezone (region of antitoxin excess in which precipitation does not occur)   The change in properties of antisera on heat treatment (65, 66, 67) or treatment with formaldehyde (9) or other denaturing agent is probably due to the con version of bivalent antibody to univalent antibody by destruction of one of the combining regions

QUANTITATIVE THEORIES OF THE PRECIPITATION REACTION   Although the quantitative physico-chemical treatment of immunological phenomena was begun early in the present century, by Arrhenius and Madsen (1, 68), it is only during the last decade that significant progress has been made   The reason for the delay is not far to seek—it lies in the fact that the mathematical treat ment of numerical data of low accuracy has little significance so long as a sound qualitative understanding of the phenomenon has not been developed.   We may mention in illustration Arrhenius' discussion (1, p 147) of the formula $C = K B^{2/3}$ which he found to express the relation between the amount C of agglutinin bound by bacterial cells and the amount B of free agglutinin, Arrhe nius interpreted this equation (whose validity for the complex system we would ascribe to the accidental distribution of the heterogeneous antiserum) as show ing that the agglutinin molecules are divided between two solvents, one within and one without the bacterial cells, and that three molecules of the bound agglutinin are formed from two of the free substance

Recent theories are of two kinds those based on thermodynamic equilibrium among the reacting substances, and those based on the rates of reactions under non-equilibrium conditions   There has been considerable discussion as to whether or not immunological reactions are reversible—whether, for example, an antigen antibody precipitate is soluble, and is in equilibrium with free antigen and antibody in solution   We know from general principles however, that, given time enough, every system reaches equilibrium, and every material is more or less soluble, the questions of interest deal rather with such quantitative points as the length of time required for the system to reach equilibrium, and the magnitude of the solute concentrations in equilibrium with the precipitate

That the precipitation reaction in some cases reaches equilibrium in the hours or days usually allowed it is shown by various experiments on solution of the precipitate by salt (69), acid (70) alkali (71), and excess antigen, even after ageing for several months (72), including experiments in which there was varia tion in the method of approaching equilibrium (19)

Experiments on the Danysz phenomenon (73) and other related experiments indicate that a long time—many days—is needed for equilibrium to be ap proached for reactions involving change in composition of antigen antibody precipitates

The first quantitative theory which we shall discuss, that of Heidelberger and Kendall (14), was based on consideration of the rate of antigen antibody combination under non-equilibrium conditions   The authors assumed that antigen A and antibody B first react completely and rapidly to form the com

plex AB, which uses up all the A (B being assumed present in excess)   There then occur two competing slow reactions

$$B + AB \rightarrow AB_2$$
$$AB + AB \rightarrow A_2B_2$$
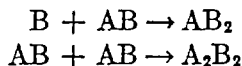
The rates of formation of AB_2 (total number of units α) and A_2B_2 (total number of units β) are

$$\frac{d\alpha}{dt} = K[B][AB]$$

and

$$\frac{d\beta}{dt} = K'[AB]^2$$

in accordance with the laws of chemical kinetics   It is assumed arbitrarily that $K = K'$ and that the reactions are not reversible, by integration over the course of the reaction until the solution is exhausted of AB there is obtained to represent the composition of the precipitate, which consists of all the AB_2 and A_2B_2 formed, the equation

$$\frac{y}{a} = 2R - \frac{R^2 a}{b_0} \tag{1}$$

in which

y = milligrams of antibody precipitated

a = milligrams of antigen added

b_0 = total milligrams of antibody

R = antibody/antigen weight ratio at equivalence point

This equation for the composition of the precipitate, which corresponds to change from $2R$ at large antibody excess to R at the equivalence point, has been shown (15) to be in satisfactory agreement with the excellent experimental data obtained by the authors   In view of the arbitrary and unlikely assumptions originally used for its derivation, it is gratifying that Kendall himself (74) has recently derived the equation in another way, and that we have found (unpublished work) that the equation is obtained as an approximation from general considerations of chemical equilibrium when the assumption is made that the ratio may vary between the limits $2R$ and R and an expansion is made in powers of a/b

Kendall's derivation is essentially the following   (He considers also some more general cases)

Let antibody and antigen both be bivalent   For B_0 and A_0 molecules of antibody and antigen, respectively, there are $2B_0$ and $2A_0$ combining groups 'Assume, for antigen excess, that all of the $2A_0$ antigen groups are bonded to antibody groups, and that they are distributed at random among the $2B_0$ antibody groups, without regard to whether or not the antibody molecule is already bonded at the other end   Since the chance that an antibody group is

bound is $2A_0/2B_0$, the chance that it is free is $1—A_0/B_0$, and the fraction of antibody molecules free at both ends is $(1—A_0/B_0)^2$. The fraction not free at both ends is $1—(1—A_0/B_0)^2 = 2A_0/B_0—(A_0/B_0)^2$, and the number not free at both ends is this multiplied by the total number, B_0. If it be assumed that all antibody molecules not free at both ends are carried down in the precipitate with the antigen molecules the molecular ratio for the precipitate becomes

$$\frac{B_{pp}}{A_{pp}} = 2 - A_0/B_0 \qquad (2)$$

and, introducing the ratio R of molecular weights, the weight ratio is found to be

$$\frac{y}{a} = 2R - R^2 \frac{a}{b_0} \qquad (3)$$

This is identical with equation 1

Similar considerations have also been used by Ghosh (75) for the derivation of related equations

An involved theory of antigen antibody equilibria based in part on probability considerations has been extensively developed by Hershey (45) The theory, in common with others based on multivalent antigen and antibody, is in qualitative and rough quantitative accord with experiment

The only theory of the precipitation reaction which, following the program begun by Arrhenius, has been developed by straightforward application of the principles of chemical equilibrium is that of Pauling, Pressman, Campbell and Ikeda (19) This theory applies only to relatively simple systems, namely, those composed of bivalent antigen and bivalent antibody, univalent hapten, certain soluble complexes, and precipitate with invariant composition AB

In order to show the nature of the treatment we present here the derivation of the equation for the amount of precipitate formed in absence of hapten generalized over the earlier treatment by consideration also of the complex AB_2

Let the molecular species A B AB, A_2B, and AB_2 in solution be in equilibrium with each other and with solid AB_{pp} We represent the concentrations of the five solutes by the symbols

$$[A] = \alpha$$
$$[B] = \beta$$
$$[AB] = s$$
$$[A_2B] = a$$
$$[AB_2] = b$$

The quantities α, β, a, and b are variable whereas s is constant for a given system with precipitate present, it is the solubility of the precipitate The equilibrium expressions for the three reactions

$$A + B = AB$$
$$A + AB = A_2B$$
$$B + AB = AB_2$$
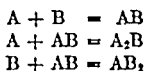
are respectively

$$\frac{s}{\alpha\beta} = 4K \tag{4}$$

$$\frac{a}{\alpha s} = K \tag{5}$$

$$\frac{b}{\beta s} = K'' \tag{6}$$

For simplicity we have assumed that each of the bonds in the complex A—B—A has the same strength as the bond in AB, this is probably a good approximation, in view of the fact that the two bonding regions are probably far apart on the large antibody molecules   (The theory can be carried through without this assumption.)   The constant K corresponds to equilibrium for one antibody valence and one antigen valence, and the factor 4 is an entropy factor or symmetry factor   We use K'' rather than K for the second bond in AB$_2$ because steric repulsion between the two antibody molecules attached to the same small antigen would be expected to decrease the stability of this complex

The expressions for the total amounts of antigen and antibody in the system (per unit volume of solution) are

$$AB_{pp} + s + \alpha + 2a + b = A_{total} \tag{7}$$

and

$$AB_{pp} + s + \beta + a + 2b = B_{total} \tag{8}$$

Subtracting equation 8 from 7 we obtain

$$\alpha - \beta + a - b = A_{total} - B_{total}$$

Eliminating β, a, and b with the use of equations 4, 5, and 6 we obtain

$$\alpha - \frac{s}{4K\alpha} + Ks\alpha - \frac{K''s^2}{4K\alpha} = A_{total} - B_{total}$$

This quadratic equation in α gives on solution

$$\alpha = \frac{1}{2(1 + Ks)} \{(A_{total} - B_{total} + [s(1 + K''s)(1 + Ks)/K$$
$$+ (A_{total} - B_{total})^2]^{\frac{1}{2}}\} \tag{9}$$

(The positive rather than the negative sign before the radical is seen to be correct by the consideration of limiting cases)   From equation 7 we find on eliminating a and b the expression

$$AB_{pp} = A_{total} - s - (1 + Ks)\alpha - \frac{K''s^2}{4K\alpha} \tag{10}$$

Equations 9 and 10 give the solution to our problem, from 9 the value of α is to be found in terms of A_{total} and B_{total} and the parameters of the system s, K, and K'' and this on substitution in 10 gives the amount of precipitate.

It is shown in the original paper how this equation (with $K'' = 0$) accounts for many observed properties of antigen antibody systems

Future progress which may be anticipated involves the extension of this straightforward thermodynamic treatment to include the case of variable composition and randomness of structure of the solid phase This will require the development of satisfactory approximate expressions for the free energy of such a solid phase, by application of the methods of statistical mechanics on the basis of sound structural concepts This problem is not an easy one, but fortunately promising methods for attacking it have been developed in recent years by able theoretical physicists interested in the problem of the stability of alloys with greater or smaller degree of randomness of atomic arrangement, and we may be confident that great progress will soon be made in the formulation of a satisfactory quantitative theory of the precipitation reaction

SUMMARY

The forces responsible for combination and attraction of antigen and antibody molecules may be classified as electronic van der Waals attraction, Coulomb attraction, attraction of electric dipoles or multipoles, formation of hydrogen bonds etc The specificity of interaction of antigen and antibody molecules arises from their structural complementariness, which permits close contact of the molecules over sufficient area for these weak forces to co-operate in forming a strong antigen antibody bond

The weight of evidence indicates that further combination of the initial antigen antibody complexes to form a precipitate is a specific rather than a nonspecific reaction and is due to a continuation of the primary combination step to form a framework structure of alternate antigen and antibody molecules

Furthermore it appears that both precipitating antigen and precipitating antibody must be multivalent at least bivalent

The more recent quantitative theories of the precipitation reaction are discussed

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PHYSIOLOGICAL STUDY OF THE VERTICAL STANCE OF MAN

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Many years ago Sherrington (1920) remarked that posture is a theme worth studying, offering much opportunity for observation by those who are willing to undertake it The literature in the field is rich and varied, spanning several branches of the basic sciences, medicine and surgery The evolution of the biped stance has received extensive anthropologic study The mechanics of standing, measurement of postural deformities, their prevention and treatment, have been subjects of comment and investigation for centuries The neurological mechanisms which control the vertical stance of man and the physiology of the cardiovascular-respiratory responses to the hydrostatic effect of gravity have been exhaustively studied Much of value to a fundamental understanding of posture may be deduced from a study of case histories which record behavior when controlling mechanisms are pathologically disrupted, or when deformity is sufficient to impair function It would require a monograph of large proportions to encompass the information available in the literature Since the subject of posture has yet to be surveyed in this way, the physiological implications of representative papers from the major fields of specialized interest form the basis of this review

The classical contributions of Sherrington (1915), Magnus (1924a), de Kleijn (1924a) and of Rademaker (1931) on the postural reflexes are well known Schäfer (1900) and Luciani (1915) have reviewed the early work on animal mechanics More recent contributions to specialized aspects of biodynamics have been made by Steindler (1935), Morton (1935), Basler (1931), Bernstein (1935), and Phelps and Kiphuth (1932) The relationships of body mechanics to health have been discussed by Goldthwait and his school (1934) A recent symposium on posture was edited by Zirbes (1938) and Hoppe (1928) has contributed a general review

According to Keith (1923), anatomists do not regard the postural adaptations of the human body as the resultant of a transformation peculiar to man, but rather as the culmination of a series of evolutionary phases which may be traced in the bodies of orthograde primates After fifty years of interest in this subject he has abandoned his original view that the gibbon is representative of the pioneers of the orthograde stock and that man and the great anthropoids had passed through a hylobatian phase of evolution (1940) He now thinks that while gibbons in their early stages of evolution were developing along brachiating lines, two other modes of orthograde posture were being evolved in the same stock In one both arms and legs were being used almost equally In the other the lower limbs were being developed as the chief organs of support and locomotion

The penalties of poor posture The change from the quadruped to the biped

posture has imposed difficulties which are interpreted by some as signs of ex treme inadequacy of adaptation (Benson 1938, Hooton, 1936, 1939)   Others while recognizing that the human body is not yet completely adjusted to the erect stance, consider the compensatory powers of Nature admirable and good (Hinchey, 1925, Main, 1937b, Jordan 1941)   It has been widely assumed that anatomical defects in skeletal alignment encroach on body cavities secondarily affecting function (Dickinson and Truslow, 1912, Cynax, 1938, Baker, 1942), and that good posture is a way to health (Canter, 1932, Forrester Brown, 1935) At the beginning of the century Keith (1903) postulated that when the abdominal wall is lax and the diaphragmatic supports give way, respiratory balance may be upset   If the inspiratory muscles gain ascendance there is danger of visceral displacement and subsequently, disorganization of function   The major in fluence on thinking in this field has been exerted by Goldthwait (1932, 1933) who has long believed that the well poised individual is the most efficient because his muscles are well balanced, and because adequate space exists for the best possible functioning of the thoracic, abdominal and pelvic viscera.

Many clinical papers in the current literature on posture indicate that stance defects may result ultimately in a variety of malfunctions including lessened respiratory efficiency, prolapse of the abdominal viscera, impairment of digestion, pressure and derangement of the pelvic organs, dysmenorrhea, hemorrhoids, varicose veins constipation, cyclic vomiting foot strain, backache, neuritis and arthritis.   Barring orthopedic disabilities few of the etiologic associations are based on demonstrable fact   Rather they are accepted as the *a priori* re sultants of a sagging chest, low diaphragm, changed position of the heart and general visceroptosis

Almost from their first enunciation these now firmly entrenched concepts have been questioned (Osgood 1916)   Many observations throw doubt on their validity   It has been pointed out that the variability of visceral position is much greater than implied by textbook descriptions of the normal (Sever, 1913, Baetjer, 1916, Barclay, 1932)   On the basis of orthodiagraphic examinations on 900 subjects Mills (1917) considers body habitus a determining factor of major importance to visceral form and position   On the other hand, Coffey (1912) thinks that the development of general visceroptosis is probably impossible in the majority of men regardless of habitus   In about 20 per cent the ascending and descending colon have not completely fused with the parietal peritoneum Coffey suggests that in this defective fifth of the human race are those in whom floating kidney mobile cecum and general visceroptosis are to be found   The position of an organ may teach nothing regarding function (Bettemann, 1916) and chronic visceroptosis may exist in the healthy (Abbott, 1934a)   Wakefield and Mayo (1937) look upon the digestive function as so fundamental an endow ment that it is beyond reason to suspect that the upright position assumed by the human species can in any way hinder hamper or decrease its efficiency   Jones (1933) represents the view between the cited extremes   He suggests that the relationship between poor posture and visceral function is "fairly definite and that by preventing bad posture one accomplishes "a little toward the pre-

vention of disease   Schwartz, Britten and Thompson (1931) assessed the stance of 2,200 normal men and boys   They came to the conclusion from the unexpected variability of the posture found in this essentially normal and vigorous group, that the importance of posture to health had been exaggerated

A few direct experimental attempts to relate posture and function have been made   Wheatly and Moore (1927) carried out roentgenographic observations of visceral position and diaphragmatic excursion on a group of young adult women, gave special corrective exercises to those below standard in postural alignment, and note what was interpreted to be an improvement in the factors studied   Klein and Thomas (1931) also reported that specific posture training resulted in demonstrable improvement of their experimental over a control group, the advantage being six to one   Schwartz, Britten and Thompson (1928) had found no appreciable change in posture, for better or worse, when nonspecific exercise was used on a test group of children   Feeling the lack of basic information, Abbott (1934b) applied the Goldthwait postural treatment to patients with asthenic habitus and symptomatic visceroptosis   He found his data difficult to interpret because of uncertainty in the evaluation of postural changes supposedly wrought by the exercises   What is needed in studies of this type is a valid and reliable criterion of good stance, adequate objective measures of anatomic displacement of viscera, and acceptable assays of the functional status of the organ systems theoretically susceptible to influence by gravitational stresses   In few case history studies have there been adequate control observations and therapy has been limited only rarely to correction of posture as the single variable under experimental investigation

Flagstand and Kollman (1928) used vital capacity as an index of respiratory damage in testing the hypothesis that functional impairment of the thoracic viscera follows lateral distortion of the spine   In mild and moderate abnormalities the vital capacity was within normal limits   In severe alignment disabilities it was reduced to within 53 and 65 per cent of normal   When the curvature was confined to the lower thoracic and lumbar regions of the spine, vital capacity was not affected even though structural abnormalities were severe   Schreiber (1934) made an experimental study on apes to observe the effects of various body positions on the shape of the abdominal cavity   He found a characteristic position of the viscera for each posture studied   Thus topographical pictures may reflect abnormality in posture rather than morphologic anomaly   In 1934 Appleton produced unusual experimental postures in young and adult rabbits   Significant deformations occurred only in the young in which growth was still active

Laplace and Nicholson (1936) initiated an extensive investigation to determine to what extent the belief that postural defects handicap physiologic function is justified by clinical and experimental evidence   They obtained orthodiagrams of the chest and electrocardiograms, measured vital capacity, respiratory rate, tidal air, pulmonary ventilation, oxygen consumption, blood pressure, heart rate, and circulatory efficiency before and after acute variations in posture   They also made a study of persons who had been taking corrective exercises for

a year Laplace and Nicholson found that the diaphragm was not always rel atively elevated nor the heart more transversely placed by postural improvement The influence of posture on the excursions of the diaphragm was variable Vital capacity generally increased There was no striking difference in oxygen consumption but pulmonary ventilation was greatly augmented The statistical significance of observed differences was not estimated They concluded on the basis of equivocal evidence that correct posture appears to have appreciable advantages to the circulation and respiration of most individuals, but that in some an apparent defect in posture may be a compensatory mechanism which it is inadvisable to disturb Subsequently Nicholson and Laplace (1938) disregard a portion of their experimental data in favor of the *a priori* view that when a good posture is maintained without conscious effort its metabolic cost is less than that demanded by faulty alignment

As a whole it has been difficult to displace the idea that attitudinal anomalies must of necessity be associated with functional disturbances It is inadequately appreciated that organ systems carry on their work within very wide margins of safety, and that many compensatory mechanisms serve automatically to protect vital processes It is difficult to believe on the basis of evidence as tenuous as that presented, that the human machine can be so fragile in its construction as to be significantly disrupted functionally by minor skeletal malalignments Indeed, Rugh (1928) recognized as did Laplace and Nicholson that though postural changes affect appearance, they may be as well signs of compensation aimed at the preservation of essential functions Möhring (1930) points out the paradoxical fact that a bad posture looks better than a good one if the so-called bad posture is balanced while the good one is not As early as 1920 Lee and Brown had introduced the term 'compensated defects." They looked upon poor posture without symptoms in much the same way as the clinician considers compensated heart disease, neglecting perhaps to take into account the vast difference in the relative importance of these two to reasonably adequate survival It has been frequently suggested that malalignment may be adequately compensated for in youth, but gives way to symptoms under conditions of strain or as muscle power wanes with increasing age

The literature following the last war makes repeated reference to the high incidence of disability developing under the rigors of military life and very generally credits faulty body mechanics as a contributory cause (Willard, 1918, Talbot and Brown 1920, Forrester Brown 1926, Milliken, 1928) Hellebrandt et al (1942) applied more precise modern methods to a re-exploration of the influence of the standard army pack on postural stability and alignment In dustry retains a concern over body mechanics in its relations to fatigue and out put (Bedale and Vernon, 1924, Zacharias 1941) but the contemporary interest of military medicine has wandered away from such problems to an intensive study of the effects of centrifugal acceleration There is a growing literature in this field It has recently been reviewed exhaustively by Ham (1943) Positive accelerations of 6 to 8 g are known to occur in the maneuvers of modern combat aviation and human subjects are being exposed to these in controlled observa

tions in planes and in the centrifuge   When one considers the amazing capacity of the human to resist centrifugal accelerations of very high order, the effects of the gravitational stresses incidental to the simple verticality of the biped sink into insignificance by contrast   That they can never be as malign as implied in a large and uncritical literature on the subject is probably a safe deduction from the type of evidence Ham reviews   Analysis of the "black-out" phenomenon will doubtless add much to our fundamental understanding of the efficacy of the various buffer mechanisms called into play in efforts to avert cerebral ischemia of incapacitating proportions   It is none the less interesting to observe that the application of simple postural tests of cardiovascular efficiency to the selection of prospective pilots was receiving interest as early as 1921 (Scott, Ellis) and that the Schneider Test continues to serve a useful purpose in aviation medicine   The capacity to resist g will doubtless influence the selection of pilots for flight training involving high centrifugal stresses   Factors affecting tolerance to centrifugal acceleration are in need of study (Rook and Dawson, 1938)

Tests and measurements of posture   Efforts to measure posture in objective ways suitable for mass examinations have been made largely by lay observers In studying the normal, they have been confronted with deviations so slight that elaborate tests have become necessary to assign grades to such variations   In contrast, precision devices have been comparatively little used by the orthopedic specialist in his examination of posture   Since the early observations of Borelli, Braune and Fischer, Vierordt and Leitersdorffer, reviewed by Schäfer and Luciani, few physiologists have interested themselves in the problems of alignment Those most active in the development of practical methods of assessing the ability of the neuro-muscular and skeletal systems to resist gravity have shown the greatest disregard for the weakness of the evidence already cited which indicts poor posture as a factor inducing malfunction and ill health   Although objective tests are more reliable than subjective ones (Cureton, Wickens and Elder, 1935), justification for making such exceedingly tedious measurements has never been put forward clearly   To date, the tests have been used largely for purposes of cataloguing and education   Few applying them have taken cognizance of the fact that "in biological terms posture is constant, continuous adaptation" and that there is a certain fruitlessness in classifying anomalies, its only satisfaction being the creation of a typology (Campbell, 1935)   Few seem to have appreciated the fact that the posture tests in current use are based almost exclusively on an untenably static concept of standing   Recognizing this point, Lee and Brown (1923) concede that the subjective rating of an examining physician who sees the body in use may give a fairer evaluation of stance mechanics than that attainable from a single instantaneous objective observation However, properly interpreted, posture tests have an obviously useful function

The literature contains a series of increasingly complex ways of testing and measuring posture   General reviews embracing methods may be found in the papers of Mott (1927), Schwartz (1927) and Babecki (1929)   To Clelia Mosher (1915) and Brown (1917) goes the credit for the earliest use of the

schematograph Fradd (1923) subsequently developed the silhouetteograph, which was later modified to include contours by Hubbard (1935), Wright (1935) and Hallett (1935) Co-ordinates were next included in the photographs (Moore 1934, Leonard 1934) to assist in evaluating the verticality of alignment Biplane stereoscopic photographs were made by Clough and Murlin (1928) and Bernstein (1930) obtained a three dimensional concept of body position with the use of mirrors

Variations in the depth of the antero-posterior curvatures of the spine have been scrutinized by the use of the lead tape, the conformature (Reynolds and Lovett, 1909), the comparagraph (Korb, 1939) and by the application of light aluminum pointers to project skeletal parts which are invisible in profile view photography (MacEwan and Howe 1934, MacEwan Powell and Howe, 1935, Wickens and Kuphuth, 1937) Although these tests yield graphic records, their rating is often based entirely upon inspectional analysis guided by arbitrary criteria influenced unduly by changing esthetic considerations Even the Mac Ewan and Howe test, which is one of the most exacting does no more than replace individual judgment by a group evaluation That this is inherent in the numerical scoring system proposed has escaped notice

All posture tests appear to be built around the assumption that the less the jointed body parts deviate from the vertical, the smaller are the rotational stresses demanding equilibration by muscular contraction and the less the energy cost The speculative nature of this view was recognized by du Bois-Reymond (1909) A vertical line coinciding with the axes of rotation of the joints of the inferior extremities and the lobe of the ear is widely looked upon as the ideal distribution of the parts around the vertical projection of the body's center of gravity This alignment, which has been called the *normal stellung* after Braune and Fischer is actually an abstraction never encountered in the normal' weight-bearing body of living man Although it is universally recognized that gravity is the main deforming force acting on the human body for the modification of posture, it is curiously true that few efforts have been made in the field of stance testing to establish an experimentally determined "weight line' as a point of departure for the analysis of the alignment of body parts This has been done by Cureton and Wickens (1935) by a method identical in principle to that of du Bois Reymond (1909) and Reynolds and Lovett (1909) Kelso and Hellebrandt (1937), and Hellebrandt and associates have synchronized biplane postural photographs with center of gravity determinations (1938 1942a, c), and used these combined methods to check the validity of one of the current methods of evaluating stance (Hellebrandt et al 1942d, e)

The biodynamics of standing The early work on *Animal Mechanics* of Borelli, the Weber brothers and Braune and Fischer is adequately reviewed by Haycraft (Schäfer 1900) Recent students in this field include Cotton (1931) Basler (1929, 1932), Okuyama (1932), Kelso and Hellebrandt (1937) and Hellebrandt et al (1937 1938 1942)

"When man became a biped and assumed the upright position he immediately made an enemy of gravity and he has been fighting this relentless foe ever

since" (Jones, 1933)   In this struggle man demonstrates some of the most striking compensatory automatisms in human physiology   Bedale and Vernon (1924) noted when several positions were taken in duplicate or triplicate on different occasions that there was nothing accidental about the postures assumed The optimum of any position could be gauged to a nicety and the versions of each mode were hardly distinguishable from each other

Reynolds and Hooton (1936) were the first to attempt an investigation of the speculative inferences concerning the relation of the pelvis to the mechanics of the erect posture   The degree of pelvic inclination with its associated lumbar lordosis is probably the most important single factor determining the postural attitude of the individual   They observed from a combination of biplane center of gravity determinations and x-ray measurements that asymmetries of the pelvis and unequal leg length bore no relation to lateral displacement of the projection of the center of gravity either in the pelvis or in the base of support

Mosher (1895, 1919) early called attention to the asymmetry of the natural stance of man   Morton (1935) assumes that the center of gravity plumbs over the geometric center of the supporting base disregarding the toes which he does not consider actively functional in normal standing   There has been interest in the exact location of the vertical projection of the center of gravity on the part of others (du Bois-Reymond, 1900, Basler, 1929, Hellebrandt et al , 1937) Hellebrandt and Fries (1942) direct attention to evidence which tends to invalidate the theoretical conception of Morton   They found that the stance of adult women was overwhelmingly asymmetric, the vertical projection of the center of gravity falling slightly to the left and behind the geometric center of the total supporting base   They propose that this phenomenon of stance eccentricity may be compensatory for a right sided limb preference

Postural sway is inseparable from the upright stance   This phenomenon has been extensively studied since the pioneer observations of Vierordt (1862) The literature has been well reviewed by Skogland (1942)   Gross estimates of the insecurity of the vertical posture were made by Weir Mitchell and Lewis in 1886   The work was continued by Hinsdale (1887) and by Bullard and Brockett (1888)   In 1893 Romberg called attention to the diagnostic significance of the inordinate postural sway evoked by simple closure of the eyes and constriction of the supporting base in patients with posterior column disease Graphic records of postural sway, called cephalograms, are made either by a stylus attached to the head or by strapping a light platform to the subject's back and having him stand under a writing point   Cephalograms have been used for the estimation of postural stability in the studies of Rosenfeld (1915), Eichkern and Skaggs (1928), Skaggs et al (1932), Skaggs (1937) and Schildbach (1940)   In 1922 Miles devised an ataxiameter which permitted better quantitative handling of estimates of postural instability than was permissible with the previous graphic methods   The ataxiameter was subsequently used by Fearing (1924, 1925a, b)   In 1940 Liebert combined the graphic and numerical methods, recording the distance covered in the cephalogram   Ricaldoni's (1928) departure in method allowed for a biplane study of the amplitude, fre-

quency, rhythm, and form of the oscillations of the body as a whole while stand
ing   He placed the subject on a platform suspended from cables   The move-
ments of the platform were transmitted to a large air capsule and thence to a
recording tambour   Moss (1931) subsequently devised a method similar in
principle and this was used by Omwake (1932)   Finally, Hellebrandt and
Kelso (1942c) obtained oscillograms of postural sway   These compounded the
shifts in the center of gravity occurring in the two cardinal vertical orientation
planes of the body during natural effortless standing

There is general agreement that stance is steadied when the eyes are open
and focussed on a fixed point and least stable with the eyes closed   Distraction
reduces sway   When the feet are together the stance is unsettled   Turning the
toes out to an angle of 45 degrees or separating the feet so as to equalize the
coronal and sagittal diameters of support steadies the stance   Sway is much
greater in the antero-posterior vertical orientation plane than in the transverse
Height and weight correlate poorly with stability   In the Romberg test the
relationships are so low as to lack significance   Thus the body may compensate
in other ways for mechanically disadvantageous factors in physical build
Though kaleidoscopic at first sight when carefully made, postural sway patterns
are characteristic for each person and highly reproducible   There is lack of
agreement chiefly as to whether stance training reduces postural instability or
not, and whether fatigue is reflected as readily as often implied, in an augmenta
tion of sway

Man possesses a relatively high center of gravity which must be maintained
over a small supporting base   Since the weight line falls in front of the ankle
joint shifting gravitational rotatory stresses must be incessantly equilibrated
One might conclude from the inseparable association of postural sway with
standing that the struggle against gravity is but poorly met   However, capri
cious as the behavior of the center of gravity may seem   its oscillations are so
accurately balanced that the average relation of the center of mass to the base of
support remains remarkably constant at least in vigorous young adults with
good equilibratory and kinesthetic senses (Hellebrandt and Fries, 1942)

The neurological basis of standing   The myotatic reflex is of exceptional im
portance to the maintenance of the upright stance (Liddell and Sherrington,
1924 1925)   The vertical orientation of the segmented body in respect to the
line of gravity is conditioned by a more or less selective distribution of this re-
flex to the extensor muscle groups   Adjuvant reflexes emanating from wide-
spread receptors teleoceptive labyrinthine proprioceptive and exteroceptive,
readily modify the geotonus of the antigravity muscles   Klein and Schilder
(1929) aptly present the postural model of the body " as one compounded from
a combination of fluctuating optic influences tactile impressions and kinesthetic
experiences   The commingled sensations which regulate postural tone are only
feebly discriminable   They rarely obtrude upon consciousness (Evans, 1926)
Fulton and Sherrington (Cowdry 1930) even place the basic proprioceptive
reflex entirely "beyond self-examination by any effort of introspection '
Although the brain has only a limited cognitive power over the muscles (Keith

1933), the acuteness of proprioception is susceptible to cortical influences (Fearing, 1925b)   However, posture is essentially an automatism and the mind is unaware of how we do our standing (Sherrington, 1933, 1941)

The orthograde stance of man is a neurological achievement of significance The multiplicity of the reflexes co-operating in its behalf suggests that the phenomenon must be important to warrant such elaborate protection   Deficiency of one reflex circuit may be associated with a compensatory coaction of those remaining (Hansson, 1932)   It is usually conceded that a kinesthetic defect accompanied by insufficiency of more than one of the accessory postural reflexes abolishes normal stance (Wiggers, 1935, Starling, 1936), but the relative importance of the various accessory stance reflexes to the normal adult human has never been definitively determined

The striking effect of retinal stimulation on the postural tonus of man is readily seen in the Romberg phenomenon   Poorly demonstrable in the normal, the magnitude of the steadying effect of visual impressions when the supporting base is narrowed, is easily appreciated when the proprioceptive reflex circuits are defective as in Fränkel's locomotor ataxia   How the eye controls the tonus of different muscle groups has been beautifully demonstrated in heliotropic insects by Garrey (1918)   Unequal photochemical reactions of the two eyes call forth asymmetrical tension, unbalanced postures, and movements along forced paths

The neck and labyrinthine reflexes have been studied by Magnus (1924, 1926), de Kleijn (1924), de Kleijn and Versteegh (1924, 1927), Beritoff (1915), and McNally (1930, 1933)   The attitudinal reflexes originating in proprioceptive receptors of the neck and in the utricle are not conspicuous in adult man, but the tonic neck reflexes are elicitable in the normal human infant during the first twelve weeks of life   Gesell (1938) considers them virtually a universal feature of neonatal infancy   Their persistence beyond the first half year indicates retarded, arrested, or defective development

Sherrington (1910) believed that centripetal impulses from the touch and pressure receptors of the soles of the feet contribute little or nothing to reflex standing   In the horse, pigeon and cat the afferent conductors could be severed without affecting reflex standing   Harris (1938) has recently described an ipsilateral extensor reflex, mild and long enduring, which may be evoked by stimulation of the medial plantar nerve in the dog   Bard (1941) describes a series of nonvisual placing reactions of importance to quadrupeds in maintaining the center of weight properly within the confines of the supporting base   Little is known about placing and supporting reactions in man   There is reason to suspect that the transformation of the weight-bearing limbs of man into rigid pillars of support by unyielding reflex tonus would be a disadvantageous retention of a primitive reaction incompatible with the maintenance of circulation in the biped

The predominant afferents responsible for the production and control of postural tonus are those of the proprioceptive group coming from the muscles and joints of the weight-bearing limbs   These are of fundamental importance

The short circuit myotatic reflexes come into synaptic alliance with the moto-neurons of the anterior horn. Their rhythmical bombardment is automatically insured by the incessant stimulation of stretch receptors incidental to the involuntary postural sway with which standing is invariably associated (Helle-brandt, 1938)   Thus streams of impulses arise within the muscles themselves While the stretch reflex is basically a spinal process (Denny Brown and Liddell, 1927), the afferent flow may impinge upon cord centers significantly modified by descending impulses of supraspinal origin which exert a profound influence on the subtetanic contractions which ultimately develop in the fraction of the antigravity motor units subjected to rotational stresses   The magnitude of the response, its speed and duration are probably related to the vigor and rate of the stretch being limited to the units specifically affected   Thus conceived, postural tonus would be automatically adapted to the force which must be equilibrated if the weight bearing limbs are to remain upright (Hellebrandt, Crigler and Kelso, 1939)

The long circuit afferent pathways mediating proprioceptive impulses travel to the cerebellum by way of the dorsal and ventral spino-cerebellar tracts and the posterior columns   Thence these impulses may be shunted rostrally by way of its superior peduncle to the thalamus and the cortex of the cerebrum or down ward to synapse with the final common path via the reticulo-spinal vestibulo-spinal or rubrospinal pathways   This suggests that the cerebellum, brain stem and cerebrum all contribute to the control of posture.   Mesencephalic transec tion between the anterior and posterior corpora quadrigemini releases the lower motor neuron from the inhibitory influence of suprabulbar centers and throws the antigravity muscles into strong tone.   The augmenting impulses emanate from the vestibular nuclei and descend by way of the vestibulo-spinal tracts

Bieber and Fulton (1938) showed that corticofugal impulses descending from the motor cortex and the premotor cortex are normally concerned in sup-pressing the neck and labyrinthine reflexes and the righting reflexes of the adult monkey and baboon   The rôle of the cerebellum in the control of postural re-flexes is imperfectly understood but Dow (1938) has demonstrated that the anter ior lobe has a well defined inhibiting effect on tonus   Bilateral destruction of the red nuclei produces characteristic disturbances in gait, mild increases in extensor and *stutz* tonus delay and exaggeration of the *schunkel*, *hinkebein* and *stemmbein* reactions   Ingram, Ranson and Barris (1934) conclude that the red nucleus is concerned with the regulation of muscle tone plays its small part as a co-ordinator and exercises restraint on certain motor activities in a way per haps subordinate to influences which originate above the mesencephalon   Met-tler et al (1939) stimulated the corpus striatum of cats and monkeys   Stimula tion of the caudate putamen or claustrum inhibited movements induced by corti cal excitation while stimulation of the globus pallidum imparted ' plastic tonus' to the cortically induced movements, exerting a holding effect upon them pro-longing their relaxation time

A variety of efferent conductors impinge on the final common path tending to increase or decrease the activity of the lower motor neurons   In ascending

the phylogenetic scale, the final common path is brought more and more under the control of cerebral centers   The basal nuclei make only indirect connections with the final common path by way of the red nucleus, the substantia nigra and the thalamus, and efferent impulses from the cerebral motor centers descend either directly or by way of the cerebellum   Thus almost all portions of the central nervous system appear to have some part to play in the control of the postural reflexes   Wilson (1926) doubts whether the skeletal muscles of higher vertebrates ever respond to afferent impulses from one source alone   Indeed, the maintenance of posture must be looked upon as the resultant of a bewildering interplay of reflexes

The chronological unfolding of the reflexes which allow for the assumption and preservation of the upright posture has been studied recently by Matulay (1938) on a group of 134 children divided into groups according to age   He found the neck and labyrinthine reflexes elicitable by the third month   Between the third and sixth months the hopping and bracing reactions appear and supporting tonus is demonstrable in the lower extremities on passive standing The tonic reflexes start diminishing during the sixth to the twelfth months and are absent in children between the age of two and one half years and five and one half years in whom righting reflexes, hopping and bracing reactions are fully developed

Under pathological conditions the primitive reflexes of infancy may reappear (Schaltenbrand, 1928)   Weisz (1938) studied the neck reflexes and equilibrium reactions as synergic phenomena   The former fade as the latter increase in strength in the course of normal development   Byers (1938) believes that the primitive postural reflex mechanisms are increasingly buried in higher mammals by the activity of phylogenetically newer neural machinery and reappear only when this is destroyed   Magnus (1926b) has expressed the view that the attitudinal reflexes seen in animals are all present in man but only exceptionally demonstrable in the healthy adult   Neck righting reflexes are present in normal adults (Schilder, 1929)   The optical righting reflexes are also active in man   Dusser de Barenne (Murchison, 1934) denies that tonic neck reflexes have been demonstrated in the normal human adult with any degree of certainty He believes that when they occur, they cannot be identified with the true neurophysiological phenomena of Magnus and de Kleijn, being rather psychogenic motor reactions   Much will eventually be learned from attempts to interpret the postural disturbances seen in clinical medicine in terms of the experimental observations of Sherrington, Rademaker, Magnus and de Kleijn (Brock and Wechsler, 1927, Haynes, 1928, Schaltenbrand, 1929, Wechsler, Bieber and Balser, 1936, Matulay, 1938, Ford and Walsh, 1940, Nielsen and Frieman, 1941)

The energy cost of standing   It has long been a favorite argument of those concerned with the correction of poor posture that so-called good body mechanics bring about a significant saving in energy and hence allay incapacitating fatigue This concept, like the supposed detrimental effect of poor posture on visceral function, is based almost exclusively on *a priori* evidence   Tepper and Hellebrandt (1938) determined the metabolism of 75 women in recumbency and

passively assumed standing   The average increase on standing was 5 71 cals /
sq m /hr or 16 25 per cent   Turner et al (1930) had observed a rise of 5 8
per cent at 62 degrees and 19 per cent at a 90 degree angle from the horizontal
These increases are small in comparison with the metabolic cost of the phasic
contractions of exercise, and could hardly be significantly affected by minor
alterations in body mechanics (Hellebrandt Brogdon and Tepper, 1940)   Lap
lace and Nicholson (1936) failed to show real differences in oxygen consumption
as a result of improvement in posture.

Sherrington (1915) called attention to the fact that postural contractions may
be maintained for long periods of time without obvious fatigue   This astonish
ing economy of postural contraction gave rise to speculation as to the cause of
such indefatigability   Hoefer (1941) concluded from action potential studies
that minimum exertion goes into standing under normal conditions   Posture
appears to be maintained by the activity of a small fraction of the motor units
potentially available   Larger action potentials occur only when weight dis
tribution is changed by shifting or swaying   From this point of view normal
standing on both legs is almost effortless

A rotation of activity among motor units has long been postulated to explain
the sustained maintenance of postural contractions without fatigue (Barbour
and Stiles, 1912 Forbes, 1922 Fulton 1926)   Bard (1941) argues strongly
against this   In part because incomplete tetani can be kept up almost inde
finitely without fatigue in mammalian muscle with intact blood supply, Bard
concludes ' there is no a priori reason for rotation ''   However if the stretch
afferents are in reality stimulated by postural sway rotation of motor units prob
ably follows whether there is a priori reason for such rotation or not   A glance
at the trajectory of the shifting center of weight during normal effortless standing
(Hellebrandt and Fries, 1942) affords good presumptive evidence in support of
checkered asynchronism of action on the part of motor units during standing and
this may secondarily account in part at least for the indefatigability of postural
contraction

Compensations for the hydrostatic effect of gravity   The gravitational force
exerted upon the circulating blood in the erect position is generally recognized as
a cardiovascular handicap by virtue of the opposition it offers the venous return
The earliest observations upon this subject were made by physicians describing
syncopal attacks   One of these, Piorry (1826), even supplemented his clinical
experience with investigative work on dogs   Physiologists long have concerned
themselves with the effects of the changes imposed directly upon the cardio
vascular system by verticality   They have given much attention to the diverse
compensatory mechanisms which exist to offset threatened disparity between the
size of the vascular bed and the volume flow in the erect position   A matter so
important for survival as the adequacy of the circulation to vital centers located
in the head is protected by a number of devices, as are many other indispensable
functions   Thus vasoconstriction acceleration of heart rate, augmentation of
respiration, general tonus of muscle, and muscular activity have all been subjects of
inquiry in this regard

There have been many publications reporting a diminution of cardiac output in normal man in the vertical position as compared with the horizontal (Lindhard, 1913, Field and Bock, 1925, Turner, 1927a, Schneider and Crampton, 1934, Donal et al , 1934, Scott, 1936, Neukirch, 1937, Sweeney and Mayerson, 1937, McMichael, 1937, Asmussen et al , 1939b)   A few observers have reported values similar for both postures or an augmentation of cardiac output during standing (Grollman, 1928, Schellong and Heinemeier, 1933, Goldbloom et al , 1940, Starr and Rawson, 1941)   The magnitude of the reduction has varied from a slight change to a diminution of 26 per cent   It is affected in particular by the length of the standing period, the nature of the change from horizontal to vertical (whether active or passive), the character of the stance (rigid, relaxed, supported or restless), and the ability of the subject to tolerate the upright position under various restricting conditions incidental to the technic of the particular procedure   In their paper, Donal et al (1934) tabulate the values for cardiac output reported in the literature   The evidence points toward the conclusion that stroke volume is significantly reduced in the vertical stance

Observations upon postural changes in blood composition and leg volume add indirect supporting evidence to the belief that cardiac output is reduced during standing   They are the consequence of withdrawal of fluid in the dependent parts combined with simple stagnation in a widening vascular bed   In 1928 Thompson, Thompson and Dailey reported an average loss of 11 per cent total plasma volume during prolonged standing   They found this to be protein free   The maximal fluid loss occurred in from 20 to 30 minutes and was returned to the circulation in about the same time upon resumption of recumbency Waterfield (1931a), using carbon monoxide instead of the dye method for estimating blood volume, obtained comparable values on the quantity of fluid lost, but observed a plasma concentration which suggested that the capillaries became permeable to protein fractions other than the globulin   His plethysmographic measurements of the inferior extremities (1931b) demonstrated volume increases of an order which corresponded well to the blood volume changes, indicating that such loss of plasma from the circulation was due to its leakage into the tissues   Turner (1930) reported a progressive increase in the volume of the legs in the erect position, as did Looke in 1937 and Asmussen and his associates in 1939 (a)   Wells et al (1938) believe the ratio of the final rate of filtration to initial rate is of a magnitude to be expected on the assumption that filtration ceases in some muscles, but continues indefinitely in others and in the skin   The distinction thus drawn between the muscles rests upon their fascial coverings   In those tightly surrounded by connective tissue sheaths, intramuscular pressures rise to 50 cm of water during quiet standing, which is sufficient to stop filtration   In those muscles more loosely covered, the intramuscular pressure reaches only 20 cm of water   Thus the latter regions may be conceived of as the filtering areas through which plasma continues to seep into the tissue spaces more or less *ad infinitum*

The work of Krogh, Landis and Turner (1932) emphasized the relationship between filtration in the capillaries and the colloid osmotic pressure by showing

that when the latter was elevated in standing, the rate of filtration produced by a given venous pressure was uniformly lower   They found a significant leak age of protein in venous stasis when the pressure became high   Youmans et al (1934) reported a marked increase in the colloid osmotic pressure of serum withdrawn from the foot during passive support at near vertical angles main tained for protracted periods of time   Keys and Butts (1939) also reported a marked increase in colloid osmotic pressure as a result of one-half hour of quiet standing and confirmed the work of Man and Peters (1933) which showed that the capillaries are impermeable to lipoids   Youmans and his co-workers found that although the leg volume increased pitting edema did not occur in any strictly normal subjects   However in spite of the self limiting effect of filtration, it appears that as Krogh Landis and Turner (1932) said, "the erect human being is constantly near to edema "

Further indirect support to the experimental evidence showing a reduction of the total output of the heart in standing is found in the observations of postural changes in blood velocity   Methods used in the study of circulation time in man include the intravenous injection of vital red (Thompson et al , 1928), of histamine phosphate (Bock, Dill and Edwards 1930), and solutions of calcium, magnesium and sodium salts (Kvale and Allen 1939, Mayerson et al 1939)   These investigators are in agreement that during verticality a retarda tion of blood flow occurs   It was more marked when the subjects were passively tilted to the vertical than when they stood, even with as little motion as possible Subsequent determinations on oxygen utilization of the legs were made by Flor kin, Edwards and Dill (1930)   They estimated that in the erect posture only one half as much blood flows through the legs in unit time as in the reclining position   Although the superficial veins fill and stretch under the stagnating fluid it is suggested that the deeper veins are less subject to change in diam eter   Kvale and Allen (1939) believe that information on the speed of flow in the arteries can be secured by subtracting the arm to-tongue time from the arm to-foot time.   The resulting figure, which gives roughly the ventricle-to-foot time, they found increased after 10 minutes in the erect position indicating slow ing of the arterial circulation apparently as a result of vasoconstriction   Ther mostromuhr measurements on dogs (Mayerson, 1942) supplement and extend these on man and provide direct evidence of vasoconstriction with a slowing of volume flow to subcardial regions   The decrease on the arterial side is ac companied by a marked decrease in venous return

The usual arterial blood pressure findings for normal erect subjects are a systolic pressure equal to or slightly above the recumbent values, and an elevated diastolic pressure   They are accompanied by a heart rate distinctly higher than that in recumbency   In 1904 Erlanger and Hooker observed that the accelera tion of the heart and the diminution of the pulse pressure upon the assumption of the erect posture remind one strongly of the effects produced by hemorrhage These normal postural responses have been widely studied under a variety of experimental procedures   In many cases the subjects have been passively tilted to the vertical to eliminate the complication introduced by the muscular work

of active change in position   The subjects have been suspended in water to counteract the hydrostatic effect of gravity   Observations have been made within 10 seconds after the change in posture and have been continued without interruption through periods prolonged to more than an hour   The subjects have been instructed to stand completely at ease, as quietly as possible, at strict attention, or have been restrained from movement by extrinsic supports   The character of the standing has not always been described   The periods of verticality have been preceded by severe exercise as well as by rest of varying duration in recumbency   They have been interrupted by periods of mild exercise such as walking, either in place or about the laboratory   Environmental temperatures have been either uncontrolled or consciously varied   More papers have dealt with the response of the male than the female

Within 10 seconds after active change from recumbency to the erect position, the systolic pressure may drop from 5 to 40 mm Hg as demonstrated by Wald, Guernsey and Scott in 1937   After about 30 seconds, they found that in the majority it regained or passed the recumbent level   The diastolic pressure usually showed a rise with the initial drop in systolic pressure, as did the heart rate   The values were exaggerated if a tilting table were used for the change in position and the systolic pressure then seldom exceeded the recumbent level When the vertical position was maintained for longer periods, the heart rate rose 14 beats or more per minute and continued to accelerate the longer the period of observation and the more quiet the subject (Barach and Marks, 1913, Sewall, 1919, Mortensen, 1923, Lutterloh, 1937, Turner, 1929, Hamilton et al , 1932, Schellong and Heinemeier, 1933, Hellebrandt and Brogdon, 1938)   The systolic pressure was not always held at its original level but frequently fell slightly The diastolic pressure gradually but consistently rose sufficiently to reduce the pulse pressure to 20 mm of Hg or less   Gross (1940) reported the oscillometric index as measured in the arteries of the lower extremities to be reduced during standing   Support of the subjects at increasing angles from the vertical has shown the arterial pressure and heart rate values to alter directly with the gravitational stress exerted on the circulation (Turner et al , 1930, Ghrist, 1930, Hamilton et al , 1932, Hellebrandt and Brogdon, 1938)   As is to be expected, nullification of the hydrostatic effect by immersion in water holds the values near those characteristic of recumbency (Erlanger and Hooker, 1904, Hellebrandt and Brogdon, 1938, Asmussen et al , 1939c)

Gravity shock—or orthostatic circulatory insufficiency   Not all individuals can tolerate prolonged quiet standing or passive verticality   As Turner emphasized in 1929, circulatory adjustment is effected by each person according to his own pattern, and it is clear that some patterns are far superior to others   Most of the investigators in this field encounter "fainters" among their subjects, even among those classified as strictly normal   They may suffer orthostatic circulatory insufficiency in from 10 to 30 minutes   Their systolic pressure is usually well maintained until just before the point of syncope   The diastolic pressure encroaches seriously upon the pulse pressure, finally falling rapidly as the systolic pressure drops   The heart rate, which may have exceeded the recum-

bent value by 40 beats per minute, suddenly slows   Schellong and Heinemeier (1933) have presented a diagram on the basis of their work with both healthy men and patients, showing the course taken by the blood pressures, stroke volume, minute volume and oxygen consumption in "excellent regulation," "adequate regulation," "the transition period," "pre-collapse," and then infer the data for "collapse" itself   Hick et al. (1940) reported a more marked postural effect in hot environments than did Hamilton et al (1932) but both groups of investigators rated the circulation less adequate in the erect position than in the horizontal

Gravity shock has been shown to interrupt standing quickly in post-exercise periods   Mateef (1935, and Petroff 1932) observed it often in sportsmen after sprints at high speed and prevented it by bandaging the legs and thighs   Brog don and Hellebrandt (1940) found it to be uniformly present in some subjects when they were immobilized in the erect position after a brief vigorous bout of work on a bicycle ergometer   The orthostatic circulatory insufficiency was often rapid in its onset   Heart rates first fell to approximately 115 per minute from exercise values near 200, then began to rise again, approaching the im mediate post-exercise level   In recumbency, post-exercise, the fall to near stabilized levels would have been steady   The diastolic pressure elevated, the systolic pressure fell far below the recumbent figure and syncope terminated the experiments

Weiner (1938) in South Africa combined the factors of exercise and a hot humid environment in studying the ability to stand   He found that most of his adult male Bantu subjects could tolerate an hour of quiet standing after shovelling gravel for an equal period of time   When, in control experiments, the standing was undertaken in a cool room, no cases of collapse occurred

Compensatory mechanisms must be highly perfected in those able to resist the gravitational stress placed on the circulation in post-exercise standing Under such conditions there tends to be a great disparity between the volume capacity of the vascular bed and the volume flow of blood   Functional dilata tion of the vessels of the inferior extremities increases their volume capacity The cessation of activity removes the well recognized muscle pump which has assured an augmented venous return during exercise   The hydrostatic effect of gravity tends to further widen the bed and oppose venous return   It is remarkable that the circulation can be maintained under such stringent circum stances

Mateef and Petroff (1932) and Looke (1937) were interested in the ability to resist gravity collapse as a problem of practical importance to choice of vocations which are carried on predominantly in the upright posture   Massee (1942) has suggested that the ideal occupation for a person with orthostatic hypotension would be that of swimming instructor, standing in water to heart level would alleviate all symptoms

Postural changes in respiration   The influence of body posture on respiration was recognized by Liljestrand and Wollin (1913)   They demonstrated an in creased pulmonary ventilation on standing   This they attributed primarily

to an augmentation of respiratory rate   Hamilton et al (1932) observed that an increase in pulmonary minute volume became evident as their male subjects reached an angle of 50 degrees from the horizontal, which is close to that found by Hellebrandt and Brogdon (1938) to be the critical position at which circulatory embarrassment commonly became evident in young adult women   McMichael (1937) denies the possibility that the increase in pulmonary ventilation is apparent only, secondary to a postural change in pulmonary capacity   The augmentation in pulmonary ventilation cannot be accounted for on the basis of increased metabolism (Turner, 1927, Franseen and Hellebrandt, 1943) Alterations in alveolar carbon dioxide also accompany postural change (Higgins, 1914, Turner, 1927, Böhme, 1937, Main, 1937a)   In trying to decide why the alveolar CO_2 falls in the erect posture, Main postulates that the drop is due largely to pulmonary over-ventilation secondary to cephalic ischemia   Hitchcock and Ferguson (1938) propose that the cause of the lowered alveolar CO_2 in the erect posture may be simple dilution resulting from the increased volume of functional residual air (Wilson, 1927), which is then maintained by an impairment of CO_2 transport from the dependent portions of the body due to stagnation of blood in the legs, but Main (1939) and Main and Baker (1941) do not concur in this view

There are many indications that postural changes in respiration are related to the hydrostatic opposition offered the circulation in standing   Turner (1927) noted that over-breathing occurred especially among subjects who tolerated protracted standing poorly   This observation is supported by McMichael (1937) who found the pulmonary ventilation/100 cc O_2 to vary approximately inversely as the absolute cardiac output

Respiratory movements are some times considered as a subsidiary line of defense against orthostatic collapse by virtue of expiratory compression of the abdomen and inspiratory thoracic suction   Hill and Barnard (1897) observed that so long as the vasomotor mechanism is intact, the splanchnic area forms what can be likened to the resistance box to the circulation, and the effect of gravity is of no importance   In the animals studied it was found that the splanchnic vasomotor mechanism was by itself sufficient to compensate for the hydrostatic effect of gravity   However, if the vasomotor tone were abolished, blood collected in the abdominal vessels, the respiratory center was then excited by cerebral ischemia and blood was pumped into the heart by virtue of the aspiratory action of the thorax and the increased intra-abdominal pressure which curbed the outflow through the splanchnic capillaries   Eyster and Hicks (1933) reinvestigated the problem on dogs and concluded that even extreme alterations of respiratory activity fail to modify stroke volume and cardiac minute volume significantly   They suggested that the importance of breathing in reference to venous return has been exaggerated   More recently Boyd and Patras (1941) have reported increases in stroke volume with inspiration which were augmented by the deep and prolonged breathing following vagotomy

Mettenleiter (1924) called attention to the influence of the pressure changes associated with deep inspiration on the quantity of blood forced from the liver

into the inferior vena cava. Edholm (1942) also believes the gasping respirations of the erect posture act along with contractions of the abdominal muscles to squeeze the liver and force blood up the vena cava. Franseen and Helle brandt (1943) describe a periodicity in amplitude of breathing during orthostatic circulatory insufficiency so marked as to resemble Cheyne-Stokes respiration Whether the increase in breathing prolonged the period of tolerance for the vertical posture through aspiratory action could not be determined The fact that the increases observed were in many cases due to rises in rate with reduc tions in amplitude renders this unlikely The augmentation seems rather to reflect a general slowing of the circulation in which the medullary centers suffer hypoxemia unless more effective compensatory mechanisms respond to combat the pull of gravity upon the blood column Franseen and Hellebrandt (1943) present evidence in support of the material benefit played by postural sway in meeting orthostatic hyperpnea

In 1937 Main postulated a proprioceptive stimulation of the respiratory center to account for changes in pulmonary ventilation during standing which exceed metabolic needs Harrison (1939) has also suggested that the increase in ventila tion produced by passive exercise or mild activity is due to a reflex arising in the moving parts and affecting the respiratory center Comroe and Schmidt (1942) likewise conclude from observations on dogs and man that the proprioceptive system definitely contributes to hyperpnea of muscular exercise Thus one might look upon postural sway as a stimulator of the respiratory center automatically regulating pulmonary ventilation in a manner destined to assist in the combat against the hydrostatic effect of gravity on the circulation There are those who also recognize the exaggerated respiratory movements of deep breathing as stimuli for a compensatory cutaneous vasoconstriction (Collier et al , 1927, Bolton et al , 1936, Lich et al., 1936), but it is purely speculative to contemplate whether or not such might contribute in any significant way to the balance of power in the struggle to avert gravity collapse.

Pathological failures in compensation The imperfections of man's adaptation to the orthograde stance have been abundantly recognized Although the literature in this field is extensive the failures in adaptation are only rarely incapacitating to a serious degree. When critically evaluated they serve con vincingly as evidence of the remarkable ability of the human machine to continue its primary functions in the face of handicaps, and to re-emphasize the buffering value of the margins of safety under which all important activities proceed Failure to isolate posture as the single etiological variable has complicated the interpretation of many reports in the literature Both the erect posture and prolonged recumbence have been shown to be conducive to the formation of renal calculi (Ward, 1037, Pulvertaft, 1939, Carlson and Ockerblad, 1940, Lich and Mansfield, 1942)

Smith and Eaton (1942) reported a series of cases in which progressive orthostatic tremor of the legs occurred during full weight bearing, while Thomas (1939) observed one in which recurrent transient paralysis of the right arm and leg could be produced by passive tilting to a 75 degree angle. Ephedrine,

benzedrine and paredrine, which controlled the associated postural hypotension, greatly diminished the number and severity of the attacks

Mosher (1909) developed an instrument for measurement of pelvic obliquity and early stressed the importance of posture to signs and symptoms of abnormal functioning of the reproductive organs in women   She reasoned that intra-abdominal pressure should be directed downward and forward toward the pubic bones and anterior abdominal wall lest the abdominal contents be forced into the superior strait of the pelvis   Lateral obliquity was associated with asymmetry in the position of the uterus   In 1917 she compared the pelvic angle of women with good and poor posture   Faulty habits of standing were associated with a reduction in the obliquity of the pelvis, increase in intrapelvic pressure and retroversion or retroflexion of the uterus   Lateral asymmetries in posture allowed the intestinal loops to slide into the pelvis on the low side and serve as a pry, moving the fundus of the uterus away from the mid-line toward the high hip

Klotz (1938) stressed the importance of proper weight lifting and weight carrying to the supporting functions of the pelvic floor   X-ray studies demonstrated a more inspiratory position of the diaphragm in incorrect lifting   This suggests that improper lifting may be associated with a Valsalva effort   Proper lifting seems to be done with the glottis open and the diaphragm in the expiratory position   That these points may be well taken is indicated by the observations of Mengert and Murphy (1934) that intra-abdominal pressures may increase from 7 7 to as high as 23 2 cm Hg by voluntary muscular effort

In 1927 Miller commenced the use of silhouetteographs in the study of the stance of gynecological patients   He postulated that poor posture produced chronic congestion of the pelvic organs which might then be the cause of leukorrhea and menorrhagia   In 1930 he reported a decrease in the incidence of dysmenorrhea with improvements in body mechanics but subsequently failed to confirm his earlier views (1934)   In 1934 Adams concluded that corrective postural exercises resulted in complete or partial relief of painful menstruation in enough cases to warrant their trial where gross pathological lesions could be excluded

Marx (1928) observed initial and final emptying time of the stomach, lying and standing   The vertical posture inhibited gastric motility   Franseen (1941) observed that gastric acidity was clearly depressed throughout and following periods of fixed standing associated with orthostatic circulatory insufficiency   With free postural sway and improvement of the circulation, the gastric secretory curves were indistinguishable from those of recumbency   She suggested that vasoconstriction in the splanchnic region, while temporarily maintaining an adequate cerebral circulation during rigid standing, might become so extreme as to impair visceral function

The pathogenesis of orthostatic albuminuria is still poorly understood   Janeway (1917) presents an historical survey of the problem   He found orthostatic albuminuria associated with lumbar lordosis   Brown (1920) and Lee (1923) noted that orthostatic albuminuria was frequently associated with poor

posture in otherwise normal young men   Beer (1937) reported 2 cases which showed massive unilateral albuminuria from the left kidney after maintenance of extreme lordosis for only 10 minutes during cystoscopic examination   He likened this to the lordotic albuminuria of orthostatic origin which is also unilateral and thought to be due to a pinching of the left renal vein between the angle formed by the abdominal aorta and the superior mesenteric artery Rytand (1937), Russell (1925) and Shannon (1942) present evidence which indicates that orthostatic albuminuria is not always "functional" and that it should not be classified as benign without careful study

Cordero and Friedman (1928) found the elimination of phenolsulphonphthalein greater in recumbency than during standing   The postural reduction ranged from 10 to 13 per cent and no correlation existed between the amount of water and of dye eliminated   They found the volume output of urine in two subjects with functional albuminuria from 50 to 200 per cent greater in recumbency than standing   Even the sitting posture reduces water diuresis by 36 per cent (Janney et al 1933)   The reduction of urinary excretory rate during standing has been postulated to be due to the number of glomerular capillaries exhibiting active circulation (White et al   1926) and to the combined effect of increased colloidal osmotic pressure and reduced glomerular filtration rate (Ni and Reh berg, 1931)   Smith (1939–40) noted a prompt reduction in renal blood flow in the majority of normal subjects tilted to the 70–80 degree angle, and failure of the filtration fraction to vary inversely with renal plasma flow   McCann (1940) estimated the diminution in renal blood flow of patients with nephrop tosis when in the erect posture to range from 20 to 43 5 per cent with relative constancy of glomerular filtration and increase in filtration fraction   This is in contrast to a reduction of less than 10 per cent in normal controls   Asmussen et al (1939b) noted that diuresis was reduced from approximately 10 cc./min to 2 8 cc /min in one subject and from 20 cc /min. to 8 cc /min in another by prolonged retention at a 45 degree angle during which oxygen consumption was practically unchanged, minute volume decreased from 4 6 to 4 liters leg volume increased by 480 cc , systolic pressure was well maintained, diastolic pressure rose slightly, heart rate was markedly elevated and serum protein increased 8 5 per cent in spite of high water intake   These investigators also demonstrated that the diuresis could be altered at will by counteracting the hydrostatic effect of gravity on the circulation by immersion in water   They concluded that diuresis was reduced by the upright posture largely through incomplete reabsorption of water, secondary to a decrease in mesenteric circulation

Orthostatic hypotension is a recently recognized syndrome related to the vertical posture   It is characterized by a chief complaint of marked weakness on standing associated with dizziness and syncope   Lowering the head, sitting or lying down are followed promptly by complete relief   The condition becomes progressively worse   Warm weather is associated with an exacerbation of the signs and symptoms   There is usually anhidrosis or hypohidrosis   The vision may be blurred   Diagnosis is predicated on the abrupt decrease in systolic blood pressure to shock levels and failure of the heart to accelerate in response to the

hypotension   Nocturia is frequently present accompanied by decrease in the urinary output in the day time if the patient is ambulatory   The deficiency in excretion of water is associated with no lowering in the excretion of phenol-sulphonphthalein   Weis (1935) observed that the total day time urinary output was 1060 cc with the patient in bed and 380 cc at night   When up and about the day urine output fell to 430 cc and night excretion was augmented to 1290 cc   Baker (1938) recorded a day secretion of 450 cc and night output of 1435 in a typical case   Croll, Duthie and MacWilliam (1935) administered a diuretic dose of water (1000 cc) and recovered 880 cc with the patient recumbent against 73 cc with the patient up and about

The first authentic case histories of this syndrome were reported in 1925 by Bradbury and Eggleston   A similar syndrome had apparently been described in about 1891 by Laubry as present in a case referred to him by Babinski   The number of recorded cases has now increased to approximately 50, most of them occurring in this country (Ghrist, 1927, 1928, Ashworth, 1929, Riecker and Up-john, 1930, Sanders, 1931, Chew, Allen and Barker, 1936, Langston, 1936, Browne and Horton, 1939, Farmer, 1941)   Several have reported cases of marked postural hypotension which have differed from the syndrome of Brad-bury and Eggleston in one major respect, that of compensatory tachycardia (Sanders, 1932, Barker, 1933, Hughes and Yusaf 1935)   It is not improbable that the two conditions are fundamentally different   Gillespie and Barker (1938), for example, observed several children with postural hypotension The manifestations differed from those already described in a number of signif-icant respects   The orthostatic hypotension was inconstant   It frequently appeared after exercise   There were tachycardia and free sweating   The circulatory insufficiency developed relatively slowly during approximately 15 minutes of standing   Gillespie and Barker credit the differences to age but the signs and symptoms suggest gravity collapse rather than orthostatic hypotension The differential diagnosis between these two phenomena has yet to be considered in the clinical literature   Gravity collapse is a physiological orthostatic cir-culatory insufficiency   Orthostatic hypotension is associated with unequivo-cally abnormal manifestations

The pathogenesis of orthostatic hypotension is imperfectly understood   The essential defect is in vasomotor regulation and the absent or diminished control of heart rate in response to the sharp change in blood pressure   Alvarez and Roth (1935) made a systematic study of the various sympathetic phenomena in orthstatic hypotension   They pointed out that sympathectomy did not produce orthostatic hypotension unless the lower thoracic anterior roots were sectioned, and suggested that this might indicate that it is the abdominal vessels which fail, not those of the lower extremities (Roth, 1937)   Kvale et al (1939) reported cases of orthostatic hypotension following anterior rhizotomy or bilateral subdiaphragmatic extraperitoneal resection of the splanchnic nerves, celiac ganglia, and the first and second lumbar ganglia in the treatment of es-sential hypertension   It may be significant that the secondary orthostatic hypo-tension in these patients was associated with tachycardia

There have been repeated signs of associated disease of the central nervous system in cases of orthostatic hypotension (Ganshorn and Horton, 1934, Ellis and Haynes, 1936, Baker 1938)   Laufer (1942) observed the syndrome in a subject with influenzal encephalitis with central nervous system residues Spingarn and Hitzig (1942) described orthostatic circulatory insufficiency in tabes dorsalis and Addison's disease, and MacLean and Horton (1937) report a case associated with myasthenia gravis   Stead and Ebert (1941) concluded that orthostatic hypotension is a selective disease of the sympathetic nervous system, probably of central origin sparing some parts   One of the 3 original cases of Bradbury and Eggleston came to postmortem examination in 1927 but the brain and cord were not studied   There was no anatomical evidence of sympathetic involvement, suprarenal disease or status thymicolymphaticus

Kvale et al (1939) made circulation time determinations in orthostatic hypotension and reported no reaction within 2 minutes when standing indicating marked stagnation of blood in the veins   Hallock and Evans (1941) reported a case of hypotension with tachycardia in which they observed an exaggeration of the normal filtration process during standing   Within 10 minutes after being tilted to the vertical posture there was an absolute decrease of 567 cc. or 11 per cent in plasma volume and 650 cc or 7 5 per cent in whole blood The normal plasma reduction in 10 minutes was noted as 80 cc   There was hemoconcentration. There were rises of 5 per cent in hemoglobin concentration, 6 5 per cent in hematocrit, and 14 per cent in plasma protein   Stead and Ebert (1941) were unable to confirm the observation of abnormal filtration rate They found that pooling of blood in the abdomen alone did not produce a striking fall in blood pressure, nor was the pooling in the extremities sufficient to account for the hypotension   When the subject stood in water, there was no pooling of abnormal amounts of fluid in the lower extremities   Stead and Ebert concluded that the essential defect in the patient with orthostatic hypotension is an abnormal response to the pooling of normal quantities of blood during standing, the definitive feature being an absence of vasoconstriction in other parts of the body when the vascular bed in one part is dilated

Corcoran, Browning and Page (1942) believe that failure of the renal pressor system may participate in some measure in the genesis of orthostatic hypotension   They tilted a patient with postural hypotension associated with tachycardia to 60 degrees and observed in addition to the decrease in systolic pressure progressing to syncope a diminution of renal blood flow   Angiotonin was then administered greatly increasing both the blood pressure and renal blood flow, in contradistinction to the renal ischemia manifest in normal subjects under like conditions   Angiotonin is the effector agent of the renal pressor system. Under usual circumstances it excites renal arteriolar vasoconstriction sufficiently to reduce renal blood flow   Corcoran et al interpret their findings to mean that in the type of orthostatic hypotension under observation the normal autonomy of the renal circulation is lost.

The subject of Corcoran, Browning and Page's observations slept for two months in the head up bed of MacLean and Allen (1940)   This brought about

a progressive delay in the onset of postural syncope Eventually, standing while doing light work was tolerated without symptoms When the patient was now tilted to 60 degrees, syncope no longer occurred and there was an increase in renal blood flow over that originally observed The administration of angiotonin was followed by a pressor response with renal vasoconstrictor effects indicative of return to normal

Sewall (1916, 1919) early tried the use of an abdominal support in the treatment of orthostatic vertigo As a whole, abdominal binders, tourniquets and bandages on the thighs have been without significant beneficial effect in the treatment of orthostatic hypotension (Barker and Coleman, 1931, Ganshorn and Horton, 1934, Alvarez and Roth, 1935) Allen and Magee (1934) reported that thigh cuffs decreased the hypotension when this could not be obviated by the use of an abdominal binder Hughes and Yusaf (1935) report a case history in which relief was obtained by walking Allen and Magee (1934) describe a patient who discovered he could temporarily avert syncope by voluntarily contracting the muscles of his legs MacWilham (Croll, Duthie and MacWilham, 1935) believes that both pulse rate and blood pressure adjustments in the standing posture are influenced by afferent impulses from the lower extremities "apparently originating in some part of the vascular circuit in these limbs " When a patient with orthostatic hypotension was made to execute a series of slight, slow movements, shifting the feet and progressing by minute steps, there was a notable alleviation of the hypotension MacWilham concluded that it was not simply the erect posture *per se* which explained orthostatic hypotension, but posture plus immobility of the lower extremities Laplace (1942) described an atypical case in which hypotension could be abolished by increasing the carbon dioxide content of the inspired air or arrest of the circulation to the active muscles The rationale of the latter may be explained in terms of the blood pressure elevating reflex of Alam and Smirk (1937) arising from voluntary muscles

Jeffers et al (1941) classified the types of postural hypotension and discussed their treatment Most success has been obtained through the use of the sympathomimetic amines on the assumption that orthostatic hypotension is associated with disturbance of the adrenergic portion of the autonomic nervous system The following drugs have been shown capable of ameliorating the symptoms ephedrine sulphate (Weis, 1935), benzedrine (Korns and Randall, 1937, Davis and Shumway-Davis, 1937, Brewster, 1940), and neosynephrine hydrochloride (Capaccio and Donald, 1938)

In 1940 MacLean and Allen described two cases of postural hypotension associated with high heart rate to which they gave the term orthostatic tachycardia One occurred secondary to sympathectomy for essential hypertension and the other demonstrated a less abrupt fall in blood pressure than usual in individuals with orthostatic hypotension That these patients probably exhibited some of the cardinal characteristics of physiological orthostatic circulatory insufficiency is indicated by MacLean and Allen's conclusion that the defect was not in arteriolar vasoconstriction but rather a failure in maintenance

of an adequate venous return. This defect in the "potential" venous return was diagnosed by the Flack test (Flack, 1923) which is essentially a Valsalva effort, the obstruction to the inflow of blood from the venous cistern of Keith by a high intrathoracic pressure being sufficient to induce syncope during the test when combined with an already defective venous return. Because the symptoms were worse upon arising in the morning than at the end of the day, MacLean and Allen postulated that something incidental to recumbency of the night was responsible for the recurrent loss of adaptation to the hydrostatic effect of gravity on the circulation. With this in mind, they placed the patient in a head up bed. The results were startlingly good. In 3 days one could stand for a half hour without syncope and in 4 days the other experienced no distress standing for one hour. Both showed return of postural syncope after one night in a flat bed. The mechanism of this unusual effect is yet to be determined.

Fine and Miller (1940) describe a bizarre case of paroxysmal auricular tachycardia which occurred invariably in sitting or standing unless under quinidine or digitalis control. It was explained by the assumption that a potential ectopic focus becomes activated in the erect posture as a result of diminished vagal activity induced by the lessened blood flow to the head which influences the center both directly and by way of the carotid sinus. In spite of the tachycardia, postural variations in blood pressure fell within normal limits. Weiss has made an extensive study of the carotid sinus reflex in health and disease. Weiss and Baker (1933) observed that pressure on the carotid sinus regularly brought on fainting more quickly when the patient was standing than when recumbent. The time interval between stimulation of the sinus and the onset of fainting was measured repeatedly in various positions of the body. It required 30 sec to bring on the attack with the head down and the feet elevated to an angle of 30 degrees, 10 sec in the horizontal and 8.5 sec while standing. The symptoms incidental to a hypersensitive state of the carotid sinus reflex almost always occur when the patient is in the upright position and are relieved by his lying down when premonitory symptoms appear (Weiss et al. 1936).

Åkesson (1936) made an extensive study of changes wrought in the electrocardiogram by the assumption of the vertical posture by individuals without evidence of heart disease or intoxication. He selected 40 cases each of the most hypersthenic and most asthenic in habitus from a group of 200 normal defense workers. There was inversion of the T_2 or T_3 wave or reduction in its amplitude in 31 per cent of the cases. The more marked the grade of asthenia the greater the change in amplitude of the T wave. In contrast there was inversion of T_2 or T_3 in 69 per cent of 45 patients with a clinical diagnosis of "orthostatic anemia." Åkesson concluded that the change in position of the heart in standing and hence in contact with adjacent conducting media could not be taken as sufficient to explain the findings. Perschmann (1939) concurs in this. T wave changes developed slowly in his subjects who were maintained at a passively supported posture for prolonged periods of time. There was no evidence of hypoxemia of the heart muscle and no relation between the blood pressure in the

vertical posture and the electrocardiographic change   There is general agree-
ment that the typical electrocardiographic change due to shift in posture from
the horizontal to the vertical is a flattening or inversion of a formerly positive
T wave in leads 2 or 3 and this occurs especially in the young of asthenic habitus
in whom there is vasomotor lability (Schlomka and Reindell, 1936, Erkelens,
1937, Hinrichs, 1937, Janzen, 1938, Sigler, 1938, Ylvisaker, 1940, Scherf and
Weissberg, 1941, White et al, 1941)   White et al believe the position of the
heart is the most important factor in the production of the T wave inversion
Mayerson and Davis (1942) suggest that the changes occurring beyond the im-
mediate ones traceable to altered heart position are due primarily to increased
sympathetic activity during the period of standing

Adaptations for the prevention of orthostatic circulatory insufficiency   The prob-
lem of compensatory mechanisms for gravity collapse was first studied systemati-
cally in the laboratory by Leonard Hill in 1895   In his classical experiments,
rabbits, cats and dogs suffered marked impairment of the cerebral circulation
when held in the vertical position   The effect was exaggerated when splanchnic
vasoconstriction was prevented   Monkeys were better able to tolerate passive
verticality than rabbits, cats or dogs, but also lost the power to do so with
impairment of vasomotor function   Vasoconstriction is considered by many
the chief form of compensation which normally occurs in the erect posture,
operating to prevent pooling of blood in the viscera and dependent limbs   In
man the diastolic pressure has been shown to rise during standing   This is a
sign of increasing vasomotor tone   It is compensatory in nature until it en-
croaches too far upon pulse pressure   The reflexes originating in the vascular
presso-receptors are responsible for this control   For some people it seems
that the carotid sinus reflex is of less importance in the maintenance of blood
pressure in the erect position that it is for others (Berry, Horton and MacLean,
1940)

There have been efforts to evaluate peripheral vasoconstriction as a factor
of compensation in the erect and tilted posture, but the results are at variance
Roth et al (1938) found an increase in skin temperature of the toes in the erect
position, in contrast to the report of Youmans et al (1935) that there occurred
a prompt and significant fall in temperature   Roth and her associates attributed
the discrepancy to the fact that the subjects of the earlier experiments were not
in a basal condition   However, Nielsen et al (1939) and Mayerson and Toth
(1939) also report evidence of peripheral vasoconstriction with passive change
of posture, confirming the conclusions of Youmans   Both made observations
at high environmental temperatures   Nielsen et al consider that the disturb-
ance of the heat regulatory function due to vasoconstriction in the tilted position
is to a certain degree compensated by an increase in evaporation   Mayerson
and Toth also present evidence that reflex vasodilatation gives way to postural
change   With heating of the extremity, the postural effect was at first overcome
until complete vasodilatation was achieved, at which point the postural change
again became evident   They found no significant differences between "fainters"
and "non-fainters" in this respect, indicating that syncope in some cases may be
due to failure of compensatory mechanisms other than vasoconstriction

Attention also has recently been drawn to the venomotor system by Weiss, Wilkins and Haynes (1937) and Wilkins, Haynes and Weiss (1937), in studies concerned with peripheral circulatory failure   The administration of sodium nitrite induced an orthostatic collapse in healthy young adults who had ex perienced no subjective symptoms when the drug was given in the horizontal position   The work demonstrates that elements of the vascular bed peripheral to the arterioles in the extremities, as well as the abdominal vessels may act as regions for the pooling of blood in the upright posture

Cardiac acceleration is a universal finding in the erect position.   Hill (1895) considered this as a subsidiary compensation   The mechanism of its production as well as that of vasoconstriction has been clarified in part with the description of vascular receptors sensitive to hypotension   Diminutions in carotid pressure, adequate to influence the cardiac reflex centers, have been shown by intra arterial determinations to occur in human subjects tipped to the vertical (Loman et al 1936)   Amussen, Christensen and Nielsen (1939) relate the rise in heart rate to volume changes of the extremities and the resultant decreased venous return of the vertical position   They considered the change of heart rate as essential in the adaptive mechanisms attempting to hold the blood pressure at levels compatible with an adequate circulation   Two summaries of their work may be found in the English literature (Krogh 1939, Asmussen, Christensen and Nielsen 1940)   The height to which the heart rate rises in standing may be considered in inverse relation to the adequacy of other compensatory mechan isms and as such is incorporated into postural tests of circulatory fitness proposed by Crampton (1913) Schneider (1920) and Turner (1927b, 1929)   In 1897 Hill, Barnard and Soltau observed that when a man is in vigorous health the effect of gravity is compensated for by the vasomotor mechanism and not by the heart.   When fatigued or neurasthenic, compensation occurs by virtue of an exaggerated increase in heart rate

Muscular contraction is clearly an influence of importance in the regulation of the circulation during exercise   The pumping effect of rhythmic compres sion and release of the capillaries and valved veins is commonly recognized as decisive in assuring the venous return requisite for an augmented minute volume during intensive physical exertion   Hill Barnard and Soltau (1897) noted in observations on healthy men, that venous tension might be grossly revealed as rising while a limb was held dependent and immobile but that the condition of the limb changed during activity of the appendage   Youmans et al (1935) showed that active muscular movements are accompanied by less concentration and less rise in colloid osmotic pressure of the blood of the active extremity than the one kept motionless during the experiment   Stead (1940) came to the conclusion that everyone will eventually faint if suspended long enough without support   He remarks that in death by crucifixion the victims were frequently tied to the cross, and the onset of collapse and syncope was hastened by the giving of wine which caused further relaxation of the vascular system

Aside from eliminating muscular activity through the use of tilting boards for passive support in verticality, little specific attention has been paid experi mentally to the slight but continual movement which is invariably present

when the nornal biped stands and which may be a decisive factor in the maintenance of an adequate minute volume in the erect posture   Its efficacy should differ only in degree from the maximal squeezing effect operative in sport and that minimal which is compatible with normality as represented by skeletal muscle tonus

Yandell Henderson (1931) suggested, in an amplification of his veno-pressor concept, that the general tone of all the skeletal muscles as well as the non-striated is a factor of great importance in venous return   In 1936, with Oughterson, Greenberg and Searle, he described a method for measuring intramuscular pressure and produced evidence of its influence on the circulation   A method similar in principle was applied by Mayerson and Burch (1940) to the study of peripheral pressure changes in men supported at a 75 degree angle   A considerable hydrostatic pressure is added to that in the veins of the inferior extremities in such a position   McIntire and Turner (1935) had found that the pressure in the veins of the foot was somewhat greater than the hydrostatic factor in healthy subjects who experienced no dizziness in a tilt to 75 degrees In one subject who exhibited poor circulatory adjustment the pressure in the veins was insufficient to overcome the hydrostatic factor   Mayerson and Burch report an immediate and simultaneous rise in venous, subcutaneous and intramuscular pressure upon change in posture   Subjects who did not develop syncope showed a secondary and usually more marked increase in intramuscular pressure during the upright period, an increase which was absent in those demonstrating circulatory embarrassment   Increasing tonus by muscular contraction eliminated all signs of syncope   The experiments of Mateef and Petroff (1934) upon subjects with pathological loss of tone showed them to be particularly susceptible to gravity shock unless protection was afforded by bandaging from sole to pelvis   Their subjects with pathologically high tone tolerated as much as 55 minutes of standing with comfort and only moderate increases in heart rate   Such observations emphasize the importance of muscle tone in preventing dilatation of the capillaries and advancing blood toward the heart

Hooker in 1911 pointed out that muscular action capable of emptying veins may be due to involuntary contractions   Many investigators have remarked since upon its possible assistance to the circulation   Mateef and Petroff (1932) noted periods of lability in the cardiovascular reactions to protracted standing which they thought might be associated with insignificant body movements MacWilliam (1933) has postulated that slight, repeated movements of the weight-bearing limbs such as very slow progression with minute steps, influence pulse rate adjustments to standing through afferent impulses, but seems to ignore the mechanical effect of such movements in aiding venous return

Looke (1937) remarked that in occupations carried on in the standing position, the slight activity of the leg muscles suffices to restrict too serious an increase in leg volume   Hellebrandt, Crigler and Kelso (1939) were able to demonstrate transient changes in intramuscular pressure during normal comfortable standing   They concluded that the rhythmic variation of intramuscular pressure was probably due to a shift in the incidence of tension between different

antigravity groups during comfortable relaxed standing   Hellebrandt and Brogdon (1938) have compared the cardiovascular response during standing with normal physiological sway permitted or restricted by extrinsic supports   Only when postural sway was unrestrained, could an hour of standing be tolerated without marked acceleration of heart rate, diminution of pulse pressure and eventual syncope   Postural sway was also shown to be a factor of material benefit in combating the orthostatic hyperpnea of normal subjects (Franseen and Hellebrandt 1943) and to serve as an adequate preventive of post-exercise gravity shock (Brogdon and Hellebrandt, 1940)

The architectural design of the human is not conducive to stability   A segmented structure, its center of gravity is placed high above a relatively small supporting base   Postural sway is inseparable from the vertical stance of man   This postural sway, autonomously controlled by the short circuit myotatic reflexes of the antigravity muscles, may be conceived of as playing a decisive rôle in the maintenance of an adequate venous return in a position as disadvantageous to the circulation as verticality   The small bulk of the antigravity muscles of the stable quadrupeds used in animal experiments can hardly be expected to play a very important part in the adjustment to vertical postures foreign to the species   Other compensatory mechanisms must be for them relatively more important   In man, however, the triceps surae, quadriceps femoris and the glutei represent an impressive share of the total muscle weight   Even partial activity of such a mass may well supply motive power against gravity to a significant quantity of blood   There is probably sufficient evidence to suggest that postural sway, acting as an accessory pump, is a more important compensatory mechanism for the prevention of orthostatic circulatory collapse than has been generally recognized   Indeed, without it, adequate venous return is difficult to maintain even in the healthy when the vertical stance must be sustained for protracted periods of time

SUMMARY

The evolution of the biped stance has been marked by a narrowing of the base of support and a progressive elevation of the center of gravity of the body as a whole   Both mitigate against stability   The vertical posture also imposes an hydrostatic handicap which encroaches enough on the adequacy of the circulation to make man in the upright stance vulnerable to peripheral circulatory collapse   The numerous difficulties seemingly attributable to the change from quadruped to biped standing are interpreted by some as signs of extreme inadequacy of adaptation   However, compensatory mechanisms automatically cancel the apparent mechanical disadvantages of the change so that gravitational stresses are counteracted easily in the majority of normal men

Posture has been studied by a variety of graphic and numerical methods which penalize angular deviations from strict verticality   The common attitudinal anomalies are widely conceded to encroach sufficiently on body cavities to impair visceral functioning   They are frequently associated with an augmentation of gravitational rotatory stresses which are assumed to necessitate ex-

haustive muscular contraction for adequate equilibration   Neither view is supported by convincing experimental evidence

The steadiness of standing is affected little by mechanical factors in build Although the center of gravity of the body as a whole shifts incessantly during relaxed and effortless standing, the patterns formed by a trajectory of the shifting center of weight and the mean position of the vertical projection of this theoretical point are relatively constant   The phenomenon of standing is elaborately protected by a multiplicity of co-operating reflexes   Primitive automatisms present in the quadruped may also be observed in neonatal infancy, after which they are submerged by phylogenetically newer controls that are capable of being lifted by disease

Gravity is the major deforming force affecting man's stance, and postural sway a constantly varying stimulus to the stretch afferents   In this way tonus is automatically adapted to the collapsing forces which must be equilibrated by the antigravity extensor muscles   The final common path through which peripheral control of standing is mediated is also subject to an inhibitory modulation by way of motor and premotor corticofugal and cerebellar paths, to excitatory regulation by the vestibular nuclei, and to less well defined influences emanating bilaterally from the red nucleus and the corpus striatum

Standing is cheap in terms of metabolic cost and it has yet to be demonstrated unequivocally that improvement in so-called body mechanics is associated with a significant decrease in energy exchange   The remarkable indefatigability of relaxed standing has not been fully explained   The sensation of fatigue undeniably associated with prolonged standing is probably the resultant of more or less acute hypoxemia of higher centers which exercise a controlling influence over muscle tonus and cardiovascular respiratory mechanisms   The hydrostatic opposition to venous return, loss of fluid into the tissue spaces of dependent parts, reduced velocity of blood flow and diminution in cardiac output act as multiple stimuli to a variety of compensatory mechanisms the objective of which is to offset the disparity between the size of the vascular bed during standing and the volume flow   The relative importance of vasoconstriction, cardiac acceleration, augmentation of respiration, skeletal muscle tone, and insensible contraction, to the maintenance of an adequate circulation during standing in man, has not been determined   Self-regulating involuntary postural sway may occupy a position in the primary line of defense by virtue of mechanical effects and reflex influences upon the vital centers located in the medulla Most of the compensatory mechanisms have been studied under conditions of graded gravitational stress, immediately after postural change, and during standing prolonged to the point of orthostatic circulatory collapse   Gravity shock is aggravated by previous exercise and high environmental temperatures, both of which augment the disparity between the volume capacity of the vascular bed and the volume blood flow

Orthostatic hypotension, tachycardia, albuminuria, oliguria, flattening or inversion of the electrocardiographic T wave, depression of the motor and secretory functions of the stomach, and dysmenorrhea have all been attributed

to the vertical stance   Failures in adaptation are occasionally so acute as to give rise to syndromes of pathological significance, but it has yet to be demonstrated that when these occur posture plays a decisive etiologic rôle

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NON-CALORIC FUNCTIONS OF DIETARY FATS[1]

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Although it is well known that dietary fats can be largely replaced by carbohydrates without any immediate and striking ill effects upon experimental animals, a sudden decrease in the fat content of human diets brings complaints from workers that they are unable to do as much work and their appetites are not satisfied Starling (177) stated in 1918 that the people lost weight rather than eat more of the high carbohydrate diet made necessary by the war-time shortage of fats Now, a new generation is faced with the same problems of fat-rationing and it seems desirable to reconsider the value of fat in the diet in the light of recent investigations The excellent review by Anderson and Williams (3) in this journal five years ago makes it unnecessary to go over the whole field again The fundamental ideas about fat metabolism are essentially unchanged and it is the object of this paper to bring the subject up to date, citing the new data which confirm or deny current theories The average American consumes about one hundred pounds of fat annually, deriving therefrom approximately one-third of his total calories This is a result of natural selection of foods according to appetite and may be considered normal Our most concentrated energy source, fats, can supply the needs for the heaviest work without undue bulk

Experience shows that the growth and reproductive performance of animals is modified by the consumption of dietary fats, which differ among themselves in their effects Since all natural fats are glycerides of fatty acids any observed differences must be attributed to 1, the acids themselves, 2, the glycerides, or 3, substances which are associated with the glycerides as impurities or as a part of the lipid molecule itself Those properties of the fatty acids, glycerides and known impurities which may conceivably account for the demonstrated effects of fats will now be considered

Digestibility It goes without saying that a food can only be as efficient as it is digestible and in this respect the average fat ranks high Many experiments with man and laboratory animals have shown digestibility coefficients of over 97 per cent It should be noted that figures in the literature are not always obtained by the same method of calculation In some cases the values are not corrected for endogenous fat (the fecal fat of controls on fat-free diet)

Some factors which affect digestibility are 1, species of animal, 2, health of the animal, 3, amount of fat in the diet, and 4, melting point and composition of the fat Method of feeding, presence of roughage and other variables, excepting high calcium, seem to have little effect on the results The present discussion is limited to digestibility in its usual sense Factors affecting rates of absorption and theories of transport across tissues have been recently reviewed by Frazer (68) and by the authors (26)

McCay and Paul (135) have shown that the guinea pig is much less able than the rat, rabbit or sheep to utilize hard fats. The rat compares favorably with the human being in this respect and by data obtained on the rat the usefulness of a fat to man may be judged.

The health of the subject is essential to utilization of fat or other foods. Obstruction of the bile duct has the most striking effects on fat absorption but other factors affect the retention of fat to a marked degree. Many people are sensitive to fats, which must, therefore, be limited in the diet. Premature infants and twins have difficulty in absorbing fats, especially the more saturated ones (185). Langworthy (108) pointed out that a number of fats, particularly beef suet and cocoa butter, exerted laxative effects on the subjects (human) if fed at high levels. Gullickson, Fontaine and Fitch (77) found that young calves suffer severe scours when certain liquid oils are homogenized into a skim milk diet. These laxative effects were not noticeable if the amount of fat was kept small. Langworthy's subjects were able to consume as much as 116 to 130 grams per day with no laxative effects. The rat tolerates fats extremely well as shown by the experiments of Hoagland and Snider (91) in which digestibilities were about the same in diets containing 5 and 55 per cent of fat.

Of all the factors affecting digestibility hardness or melting point has received the most attention. There is a point above which fats are so poorly utilized that they are not only lost as food but also exert deleterious effects upon the animal. Instead of the 97 per cent digestibility of liquid oils and soft fats the utilization of harder fats, either natural or synthetic, is likely to fall gradually as melting points rise above body temperature, until at 50°C only 90 per cent retention by man may be expected. With strictly comparable triglycerides the effect of melting point can be strikingly demonstrated with rats. For example, Evans and Lepkovsky (64) found the following digestibilities in per cent for palmitin (m.p. 58-60°C.), myristin (m.p. 53-54°C.), laurin (m.p. 43°C.), caprin (m.p. 25-26°C.), and caprylin (m.p. 7-8°C.), respectively, 73.4, 91.0, 96, 99.5 and 97.5. It will be seen that an important loss in digestibility has occurred at a melting point of 53°C. In an attempt to correlate digestibility with the melting points of mixed fats Hoagland and Snider (92) studied a group of lards, hydrogenated vegetable shortenings and shortenings made of mixed animal and vegetable fats. In the range from 39°C. to 56°C. no correlation was found between the percentage utilization and the melting point, or amount of total saturated acids in the fats. There were, however, large differences in digestibilities as shown by the following data: average for lards 94 per cent, average for hydrogenated vegetable shortenings 87.5 per cent, and average for vegetable and animal shortenings 85.0 per cent. These are significant differences for which an explanation may be found in some of the following facts. The excreted fatty acids are of very low iodine number, showing a selective excretion of the saturated acids which must, therefore, to a considerable extent affect digestibility. Holt and co-workers (96) were able to predict the digestibility of fats by infants by assuming the following percentage retention of the fatty acids: Unsaturated, 98; short chain saturated, 90; palmitic, 88; stearic, 60; and saturated acids longer than stearic, 40. Barbour (8)

found the arachidic acid (m p 76°C) of peanut oil almost wholly excreted in the feces along with appreciable amounts of stearic and palmitic acids   Hence digestibility depends more upon the amounts of longer chain saturated acids than upon the total saturated acids   Hydrogenation may have produced stearic acid without increasing the total saturated acids beyond the value for the lards, which are much richer in the more readily absorbed palmitic acid

One other point is of interest here   The same acid in compounds of different melting points will be absorbed to different degrees as shown by the ready utilization by rats of ethylstearate (m p 30–31°C) as compared with stearin (m p 71–72°C) (64)   However, there are numerous reports of poorer utilization by other animals of ethyl esters as compared with the glycerides (96)   The melting point of a mixed fat is not a good criterion of its composition   A difference of several degrees in melting points of glycerides arises from a shift of a fatty acid from the α to the β-position (41)   The decrease in in vitro hydrolysis by pancreatic lipase of hydrogenated fats of increasing melting points correlates well with the observed fall in digestibility (186)

It is evident from the cited data that the hardness of fats and their content of long chain saturated acids exert a material effect upon their food value   Melting point, as determined by the official methods, is not an exact indicator of digestibility of mixed fats, although in a broad sense its effects are evident   If for no other reason, it is a mistake to hydrogenate fats so completely that their digestibilities are materially affected

Mobilization and utilization of the fatty acids   It has long been known that dietary fats are deposited in body tissues, milk, and eggs   This occurs so extensively that it can hardly be said that any fat is characteristic of a species, unless it be that synthesized from non-fat precursors which McAmis, Anderson and Mendel called "physiological" or "synthetic" fat (133)   The appearance of dietary fat in milk was demonstrated by Stohmann in 1869 (180), in body fat by Lebedeff in 1882 (112), in the fetus by Thiemich in 1905 (184), and in eggs by Henriques and Hansen (84) in 1903   The many recent papers, by furnishing more detailed information about this phenomenon, have shown that the results obtained depend upon 1, the body tissue or fluid analyzed, 2, the species of animal, 3, the kind of fatty acids used, and 4, the method of conducting the experiment   The sum of the evidence from these "tagging" experiments demonstrates the lability and rapid turnover of tissue lipids, a result confirmed and extended by the very recent use of isotopes and isomers

The method of experimentation may largely control the absolute results Starvation followed by refeeding brings about the most rapid change in body fats High levels of fat in the diet produce in a short time the same result as low levels fed over a longer period   Some tissues are much more affected than others Blood and liver lipids reflect almost immediately a change in dietary fat   More slowly the adipose tissue may change several fold in iodine number while the brain is exceptionally constant in its fatty acid content (171) (170), a stability possibly related to the inability of nervous tissue to burn fat or acetone bodies Although phospholipids may reach a high degree of unsaturation, their percentage

change in iodine number is not equal to that which may be induced in the simple glycerides (168)   Dietary fats produce changes in egg (39) (136) and milk (86) lipids which do not closely parallel the changes in depot fat (163) (93) (93)   This selective utilization of fatty acids varies so much with the species that it may be considered a "species characteristic"   Certainly it plays a large part in fixing the composition of the body lipids of the different animals on their natural diets   The pig, rat and chicken are very responsive to dietary fats, their body lipids being changed profoundly by the diet (117) (54) (2) (39) without apparent effects upon their metabolism or well being   In contrast, the body fat of beef and sheep (90) remain fairly constant in composition regardless of diet

In those animals which respond to dietary fats a high degree of selection of individual fatty acids takes place, some being excluded from the tissues while others are preferentially held   This preference for certain acids is based upon properties associated with chain length (molecular weight), unsaturation, or melting point   It is not essential that the fatty acid be one usually found in diets or that it be the naturally occurring isomer   Erucic acid, an uncommon member of the oleic series, was found by Munk (140) to be deposited in the body fat of dogs   McConnell and Sinclair (137) were able to introduce into the lecithin and cephalin of the rat nearly thirty per cent of elaidic acid, the high melting trans isomer of oleic, which Barbour (7) had already shown to be readily stored in the whole body fat (9 2 per cent) and utilized in starvation like other acids in his study of the nutritive value of hydrogenated shortenings   The eleostearic acids (conjugated isomers of linolenic) are readily incorporated into tissue lipids of the rat (143) and hen (40) and secreted unchanged in the milk of the cow (97)   The conjugated isomers of linoleic acid (142) and brominated acids (4) are other un natural forms which react in the body enough like the natural ones to make them useful as tracers, a subject recently reviewed by Bloor (14) and Chaikoff (33)

Since butter fat and coconut oil are among the best food fats it is obvious that the low molecular weight acids are readily utilized when taken as a part of a mixed diet   However, a high degree of selectivity is shown by animals in their utilization of fatty acids of different chain length   In 1882 Lebedeff (112) showed that neither butter nor tributyrin in the diet increased the volatile acids of the body fat of the dog   Since that time much work has been done to estab- lish the fact that fatty acids with less than 10 carbons are not deposited in more than traces in rat body fat (51, 50 42, 154, 153, 37, 98, 34, 166, 118, 114)   A very sharp break occurs at this molecular weight, the records showing only traces of caprylic (154), 15 per cent of capric and 25 to 32 per cent of lauric (153) (114) acids in body fats of animals fed in comparable ways   These very short acids must be immediately degraded (166) or synthesized into the longer acids and stored   There is considerable evidence for some synthesis and deposition of the higher saturated acids by addition of two or more carbons (51) (154) (166)   Thus the low molecular weight acids may affect the body composition without them selves being deposited   Immediate degradation of the major portion of these acids has been demonstrated by the use of the deutero-compounds (166) as well as by their ketogenic action even when the liver is rich in glycogen (121)   A

marked rise in blood acetone bodies follows ingestion of the lower fatty acids (C_4 to C_{10}) but not the higher (C_{12} to C_{18})   Another interesting observation is that caproic and butyric acids do not prevent the rise of blood sugar and the loss of galactose from the urine of rats on a skim milk diet while the higher fatty acids and many common oils bring about the normal utilization of galactose (165)

Body fats of higher animals made by synthesis from carbohydrate and protein are comprised chiefly of oleic, palmitic and stearic acids   Usually about 95 per cent of the acids have 16 or 18 carbons (54) (88) (116)   The strong tendency for the maintenance of this high percentage of these two groups is well demonstrated by the work of Hilditch and Longenecker (87) in which the body fat of milk-fed rats is compared with the milk fat   Of 22 8 molar per cent of C_4 to C_{12} acids in the butter only 1 4 molar per cent was found in the body fat   There was a good storage of myristic acid   By a forced feeding of large percentages of the 10 and 12 carbon acids it is possible to have them deposited in quantity and upon starvation they are readily utilized   In fact Longenecker (114) finds a greater rate of loss of lauric than other acids from depot fats very rich in it   This might be interpreted as selective utilization but, as he points out, it may be determined by the glyceride structure   There is very little evidence for much selective use of the ordinary depot fatty acids on starvation of pigs, rats, sheep and other animals although small differences have been regularly observed (89) (90) (115)   As Hilditch and co-workers point out, the general distribution of the fatty acids in mixed glycerides militates against use of a single acid if it be assumed that the whole glyceride must be utilized as a unit

Unsaturation of a fatty acid exerts a marked effect upon its deposition   The higher animal, which is incapable of synthesizing more than traces of the highly unsaturated acids, except arachidonic, may preferentially retain a large proportion of those present in the diet (53) (2)   Much of the linoleic acid of the feed is retained in the body of the pig (53)   This acid is considered largely responsible for the softening of lards   When 60 per cent of the calories of the diet come from fat the iodine number of the body fat of rats follows closely that of highly unsaturated oils (2)   When more saturated fats, lard and Crisco are fed there is a selective retention of the unsaturated acids which makes the body fat more unsaturated than the dietary fat   Exceptional in its effects is cottonseed oil which, upon inclusion in the diet, raises the melting point of hog lard (55) and of cow's milk fat (49)

The very highly unsaturated acids of fish oils are avidly held by certain tissues, a property which probably led to the theory of desaturation (167, 169, 168, 86)   The phospholipids, especially sensitive to these acids, will show a marked change in fatty acid content with the inclusion of only a trace (50 mgm per day) of cod liver oil in the diet of the rat (168)   The change takes place quickly, being complete within 10 to 15 days (169)   Their affinity for unsaturated acids is so great that phospholipids pick them up from coconut oil or butter diets which markedly lower the iodine number of the neutral glycerides (167)   Linolenic acid does not seem to be so readily deposited in the rat (167), nor is it secreted in appreciable amounts by the mammary gland of the cow which does transfer much of the fish

oil acids into the butter (86) It is, however, readily deposited in egg yolk as are the acids from sperm oil (39) (40) In general, saturated acids in the diet do not so markedly affect the composition of body fats, eggs or milk Barbour (8) concluded that, possibly due to limits of digestibility, the saturated acids of rat body fats cannot be raised above the "normal" of about 25 per cent Cruickshank (39) also found relatively little effect of ingested saturated acids on egg fat It seems that this rule should be applied only to the longer chain acids When butter, coconut oil or the C_{12} or C_{14} acids themselves are fed the effects are very definite Fasted rats, fed a diet rich in coconut oil, may deposit as much as 72 5 molar per cent of saturated acids in the depot fat (118)

In addition to being deposited or immediately oxidized, ingested fatty acids may be saturated (hydrogenated), (143) (40) (119) (166), desaturated, partially degraded to normal acids with fewer carbons (86) (114) or lengthened to a higher molecular weight (51) All of these changes, postulated from analytical data, have been given direct proof by the use of deuterium Desaturation of stearic and palmitic acids to oleic and palmitoleic, respectively, has been demonstrated but linoleic and linolenic are not formed in this way by the animal Nor do they take up deuterium from the heavy water of body fluids, proof that they are not synthesized by the rat or mouse but must be derived from the diet

The essential fatty acids This subject recently reviewed in great detail by one of us (24), will be treated more briefly here and brought up to date by the addition of some new references To quote from that review "The term essential fatty acids was introduced in 1930 (29) and since that time has been used rather loosely to designate a group of unsaturated acids which will bring about a renewal of growth of rats which have reached a plateau on a low fat diet Strictly speaking only one of these is essential If linoleic acid is added to the deficient diet, growth will be renewed"

A large number of purified fatty acids have been tested in this way in different laboratories and it has been demonstrated that they differ in their effects upon the animal Exclusion of fat from the diet leads to 1, development of scaly skin and caudal necrosis, 2, retardation of growth, 3, kidney lesions and hematuria, and 4, early death Males become sterile and females produce poor litters and fail in lactation High respiratory quotients high metabolic rates and high water consumption have been observed and histological changes in various tissues described

Of the above effects growth response and skin cure seem to be the best measures of effectiveness of oils and fatty acids In some instances (62) (187) the skin is only mildly affected but this is probably due to unusually high humidity in the colony (24) The effects upon the oestrus cycle are irregular (187) and in young animals may not show up at all (156) Those acids which have been reported to have some curative effect upon fat deficient rats are linoleic, linolenic, arachidonic, decosahexenoic and hexahydroxystearic In addition, linoleyl alcohol is useful All other fatty acids and isomers of the above compounds have been reported negative

Linoleic, arachidonic and linolenic acids are the only ones which have been

extensively worked with   Quantitative comparisons of their growth effects, made in different laboratories, are not in close agreement but it is clear that linoleic and arachidonic are much alike while linolenic is inferior   This acid produces some growth but does not cure the skin, reduce water consumption to normal or improve lactation (24) (156)   Decosahexenoic acid and the mixed esters of cod liver oil react in a similar way and it can be said that linoleic and arachidonic acids are highly specific in their effects upon the skin

The rat is the only species studied sufficiently to establish the daily requirement for linoleic acid   Prophylactic doses of 20 mgm gave optimum growth in young rats (128) (28), 23 mgm gave good growth and reproduction (124), but for optimum lactation 100 mgm is recommended by Quackenbush, Kummerow and Steenbock (156) because the performance of their rats was superior to that reported by earlier workers   In curative experiments the higher levels of 50 to 100 mgm daily seem distinctly superior (187) (27) (30) and it must be concluded that the lower requirements for early growth of young well-fed rats (92) is due to the stored linoleic acid which is limited by the diet of the mothers (25) (158) (157)   This effect is so pronounced that the average longevity of rats on a fat deficient diet may be increased from 4 months to 14 months by changing the mothers from coconut oil to corn oil (25)   Also, the iodine number of the carcass fats of weanlings may be reduced from 134 to 94 (158) by changing the mothers from stock diet to a diet made of potato meal, casein and salts

Very few experiments have been done on the essential fatty acid requirement of other species   The goat, cow, chicken and pig have been kept for short periods on diets low in fat without the development of outward signs of the deficiency However, the mouse responds to a lack of linoleic acid (193) very much as the rat does except that in combined pyridoxine and linoleic acid deficiency it declines and dies before developing either scaly skin or acrodynia (67)   When reared on a simplified low fat diet similar to that used for an experiment with man (21) dogs develop scaly skin which is curable with fats (81)   The iodine number of the blood lipids drops about 25 per cent and rises as the cure takes place

Little is known about the requirements of man for unsaturated fatty acids Two infants maintained by Von Gröer on a diet very low in fat grew fairly well but one developed a generalized eczema (189)   Of three infants kept on a fat-free diet by Holt and co-workers (96) one developed an eczema which was cured by feeding fat   In two infants maintained at the University of Minnesota Hospital for 10 weeks on a diet extremely low in fat there was a moderate decrease in the iodine number of the serum lipids   An adult human subject maintained for six months on a diet which produces typical fat deficiency symptoms in rats felt no ill effects but he lost weight, suffered a change in respiratory metabolism and a marked fall in the arachidonic and linoleic acid content of the serum lipids (21)

The above effects parallel closely the changes in rats suffering linoleic acid deficiency   A distinct fall in blood iodine numbers accompanies the development of scaly skin (80)   Assuming an analogy between rat scaly skin and simple infantile eczema Hansen and co-workers (79) began a study of eczematous human subjects, in whom they have found a distinctly lower blood content of

arachidonic and linoleic acids (20)   In infants with uncomplicated eczema the average fall in serum lipid iodine number is about 25 per cent (79)   When corn oil, lard or other unsaturated fats are fed most of the eczemas are cured or greatly alleviated   About 50 per cent of hospitalized adult patients with subnormal serum fatty acid iodine numbers showed distinct improvement of their skin when treated with lard by Finnerud, Kesler and Wiese (66)   Although the results with human subjects are not as uniform as with experimental animals under controlled conditions, it seems clear that many people are receiving less than their optimal requirement of linoleic acid   From a survey of the national dietary it has been estimated that linoleic acid constitutes about one per cent of the average American food   It is likely that many people consume less than this amount, which has been suggested by some as the requirement for rats   It is not surprising, therefore, that there are individuals who, because of low intake or abnormally high requirement, respond to additional unsaturated fatty acids in their diet

That the most marked human response should be in the skin falls in line with the view that linoleic acid is highly specific for that tissue   The first symptom of its deficiency is scales on the feet of young rats which may appear within 10 days and before growth has been measurably affected   The thicker and more differentiated skin of fat-deficient rats has been recently described by camera lucida drawings (195)   Possibly the most striking effect of linoleic acid on the skin is produced by a combined deficiency of pyridoxine and fats   Severe acrodynia is produced which can be completely eliminated by the feeding of linoleic acid   When fed at sub-optimal levels pantothenic acid and linoleic acid supplement each other but only linoleic acid can alone cure all skin symptoms (159)   In an attempt to explain these inter-relations the body lipids of the rats used in the above experiments were analyzed (158)   Some differences were found in the fat content and iodine numbers of the groups but there is no evidence that pyridoxine or other B factors bring about a synthesis of linoleic acid

The ready mobilization of the unsaturated fatty acids is shown by the work of Smedley MacLean, Hume and Nunn (175) (174) (176) who find that the arachidonic acid of subcutaneous tissue is rapidly moved to the growing liver and developing tumors   Tumor growth was not retarded by limiting the intake of unsaturated acids

A new specific function of linoleic has been described by Engel (56)   With synthetic vitamin supplements and a fat-free diet, choline had its optimum lipotropic effect only when inositol, pyridoxine and linoleic acid were all present   One-tenth milliliter of corn oil daily reduced the liver fat to half in one experiment.   The author postulates a mutual influence of these factors in the mobilization of fat, although it is known that linoleic acid deficiency does not materially decrease the rate of incorporation of labeled fatty acids into liver phospholipids (0)

The relationship between dietary fats and other nutrients   Fats markedly affect the availability and utilization of a number of dietary essentials   It is difficult to evaluate the importance in human nutrition of these effects which are readily demonstrated with laboratory animals   In certain cases, particularly when oxi

dized or rancid fats come into intimate contact with other foods either in the
dietary mixture or in the intestinal tract, the relationship of fats to other nutri-
ents assumes an increased significance   In the following discussion an attempt
has been made to bring together some of the pertinent information on the effects
that both fresh and rancid fats have on other dietary substances

Vitamin A   It has long been recognized that different fats have a profound
effect upon the biological efficacy of vitamin A and its precursor, carotene   Only
one year before Moore's (144) discovery that carotene is converted into vitamin
A in the animal body, Duliere, Morton and Drummond (45) found that carotene
which was dissolved in ethyl oleate was biologically inactive and reported that
carotene did not exhibit vitamin A activity   Even before this time it had been
observed that vitamin A was poorly utilized when administered in mineral oil
(46, 31, 140) and following this that the utilization of carotene was even more
affected (47)   Natural fats were shown to have varying effects upon carotene
utilization and in 1935 the Medical Research Council concluded that in vitamin
A assays the standard β carotene was more efficacious if dissolved and admin-
istered in coconut oil or certain samples of arachis oil than when given in olive oil,
hardened cottonseed oil, ethyl laurate, or liquid paraffin (139, 109)

It is probable that at least two separate factors are responsible for the diverse
effects of fats on vitamin A and carotene utilization   These are 1, absorption,
and 2, oxidative destruction   Largely through the well known work of Drum-
mond, Bell and Palmer (44), and Greaves and Schmidt (75), it is now known that
to some extent both carotene and vitamin A follow the general pathway of fat
absorption   Furthermore it has been shown by Ahmad (1) and Mulder and
Kelly (145) among others that dietary fat is necessary for the normal absorption
of vitamin A and that with amounts up to at least 10 per cent of the total weight
of the diet, the apparent absorption of the vitamin increases

It is probable that the loss of biological potency, apparent when the vitamin
or its precursor is administered in various natural fats, is largely due to oxidative
destruction before absorption takes place   This oxidation might be brought
about by simple diffusion of atmospheric oxygen through a solvent which in itself
neither accelerates nor inhibits the reaction   However, the formation of pro-
oxidants which increase the rate of carotene destruction is of more serious conse-
quence   Baumann and Steenbock (11) found that carotene dissolved in ether,
chloroform, cyclohexane, acetone, carbon disulfide, toluene and pyridine, and
kept at 4°C was more than 75 per cent destroyed in 5 months while other solvents
such as benzene and petroleum ether were less destructive   Ether, the solvent
which caused the greatest loss, readily becomes contaminated with peroxides

In the above study, hydroquinone effectively reduced carotene destruction in
all of the organic solvents that were employed   In 1931 Olcovitch and Mattill
(148) came to the conclusion that the stability of carotene in solution is depend-
ent upon the nature and the amount of antioxidant present   The importance
of antioxidants was further stressed by Shrewsbury and Kraybill (173) who found
that if butter fat was treated with charcoal the vitamin A and carotene, together
with natural antioxidants, were destroyed and furthermore that if carotene was

added to this charcoal treated butter fat and immediately fed to rats an appreciable part of its potency was lost. If hydroquinone was also added, the carotene was protected against *in vitro* oxidation but it still lost a part of its biological activity (106). A number of results have been published on the biological potency of carotene dissolved in different fats and in many of these no correlation between *in vitro* oxidation and biological potency could be observed. Sherman (172) clarified some of the discrepancies in earlier publications by showing that there are real differences in vitamin A potency dependent upon the fat in which it is administered, but that this effect is observed to a noticeable extent only when the basal diet is fat-free except for the essential fatty acids.

Another demonstration of the influence of antioxidants on vitamin A potency was recently presented by Hickman, Harris, and Woodside (85) who used the U S P vitamin A assay method with olive oil as the specified vegetable of the basal diet and studied the effect of added vitamin E (molecular distillate containing 40 per cent mixed tocopherols) on the growth response to fixed quantities of three different forms of vitamin A (crystalline alcohol, a crystalline ester and mixed carotenes). With all three substances the addition of sufficient vitamin E markedly increased growth. In the case of carotene, a dosage of the vitamin that was insufficient to support life if given alone, induced good growth when fed with sufficient vitamin E.

It was observed that the tocopherols were effective only if administered simultaneously with the vitamin preparations. This would indicate that they protected the vitamin A from oxidative destruction while in the intestine. Baumann, Rusing and Steenbock (10) showed that when a butter fat concentrate dissolved in cottonseed oil was administered to rats no storage of vitamin A in the liver took place to a large extent and when losses due to destruction in the intestinal tract were large during the intestinal losses were small so that faulty absorption could not account for this intestinal loss. If intestinal destruction of vitamin A is largely prevented by vitamin E, hydroquinone which is a much more potent antioxidant than vitamin E might be expected to have a greater effect. However, it seems from Kraybill and Shrewsbury (106) and Quackenbush, Cox and Steenbock that this is not the case. The latter investigators using both a cottonseed oil and α-tocopherol together with carotene dissolved in ethyl laurate (iodine value = 0) have come to the conclusion that hydroquinone, being a water soluble compound, is extracted from carotene solutions in the intestine and therefore cannot exert any antioxidant action at this site. On the other hand α-tocopherol being fat soluble remains with the carotene and gives it protection during its passage through the intestinal tract ... ... at,

With the development of rancidity *in vitro*, natural antioxidants ... and low and carotene and vitamin A are destroyed. The substance which causes this destruction is still unknown. Lease, Lease, and ... the the (111) in an extensive study on this subject have come to the ... the sions. Vitamin A is not destroyed by oxidized glycerol, glycerol ... ut 1 hyde, allyl alcohol, straight chain aldehydes, m ...

or decomposition products formed by the commercial hydrogenation of fats
The components of rancid fats which cause destruction of vitamin A are not re-
moved by steam distillation nor by extraction with alcohol From the vast
literature that has now accumulated on this subject of *in vitro* vitamin A oxida-
tion it would appear that loss of activity is associated with the intense peroxide
formation that follows the termination of the induction period of the fat in
which the vitamin is dissolved Sumner (181) has illustrated this relationship
between rate of peroxidation and carotene destruction by using active lipoxidase
preparations It is his conclusion that intermediates of fatty acid peroxidation
are the active agents involved, but not the peroxides themselves

Vitamin D It was early pointed out by Zucker and Barnett (196) that dietary
fat exerts some antirachitic action and this observation has been amply substan-
tiated by more recent investigations (104) (105) (138) (15) (76) (103) (100) (101)
(17) The antirachitic effect of fat in the diet is relatively small but consistent,
and is dependent upon the amount of fat present For example, Knudson and
Floody (103) found that with 0 025 mgm of crystalline vitamin D_2 dissolved in
propylene glycol and added to 40 grams of a rickets producing basal diet, the
extent of healing was about 3 times greater if 5 per cent hydrogenated vegetable
oil was included With 10 per cent fat the vitamin D was less effective and 20
per cent reduced the healing to the approximate level of the fat-free basal diet

Explanations of this antirachitic effect of dietary fat have been largely con-
cerned with the influence that fat might have on calcium and phosphorus absorp-
tion It is known that in sprue, celiac disease and in other types of profound
steatorrhea, calcium absorption is impaired, the unabsorbed calcium being ex-
creted in the feces, for the most part, as calcium soaps It has also been ob-
served that when the fat content of the diet is greatly increased the amount of
calcium soaps in the feces increases (38) (5) (160) (13) However, it has been
often reported that small amounts of fat in the diet have a beneficial effect upon
calcium absorption (94) (95) (19) (70), but it is not fully agreed that fat increases
the absorption and retention of calcium or the calcification of bones (17) under
all conditions It would appear that both calcium absorption and calcification
are more definitely enhanced by dietary fat in the rachitic animal than in the
normal

In an attempt to explain the manner in which vitamin D-free fats influence
calcification Jones (101) studied the effect of a number of substances on the
acidity of the intestinal contents and on the calcification of the bones of rachitic
rats In agreement with the work of others it was found that fat (10 per cent
lard or oleic acid) decreased the pH of the lower portion of the small intestine at
the same time showing a definite antirachitic effect However, lactose also
increased the acidity of the lower intestinal tract, but did not exhibit any anti-
rachitic action Dibasic phosphate protected the rats against rickets without
changing the pH of the intestinal tract Although these experiments do not
completely rule out intestinal acidity changes as a factor involved they do point
out the obscurity of the mechanism of the fat effect on calcification

Vitamin E Early in the development of the knowledge concerning the physi-

ology and chemistry of vitamin E it was pointed out that amounts of wheat germ oil which added to a fat-free diet were sufficient to relieve sterility in rats became ineffective if lard, crisco, or oleic acid were included in the diet (60)   The explanation for this effect of fat on vitamin E activity, simultaneously presented by Mattill (129), and Evans and Burr (59), was that oxidative changes which accompany the development of rancidity in unsaturated fats destroy the vitamin Macomber (125) added 5 per cent wheat germ oil to a diet containing 20 per cent lard and found the diet to be deficient in vitamin E

Goettsch and Pappenheimer (73) first pointed out that rabbits and guinea pigs developed a characteristic muscular dystrophy when fed diets that were treated with ferric chloride and contained lard and cod liver oil   The ferric chloride treatment was known to destroy vitamin E (190) and the lard and cod liver oil additions seemed to aggravate this deficiency symptom   Madsen (126) found that muscular dystrophy could be produced by a vitamin E-free diet containing a cod liver oil concentrate without lard being present   The addition of cotton seed oil afforded some protection, but if cod liver oil in its natural form was included, cottonseed oil was without effect   In view of the fact that muscular dystrophy can be brought about by the ingestion of cod liver oil concentrates as well as by the natural cod liver oil it is interesting to note that Evans and Burr (59) had found that the vitamin E destroying material of oxidized lard could be concentrated in the unsaponifiable fraction

Although it is not fully agreed that the sole reason for the development of muscular dystrophy in certain animals fed cod liver oil is through a destruction of vitamin E, there is an accumulation of evidence that the dystrophy can be prevented by the appropriate administration of α tocopherol (123) (122) (57) (130)   It is the opinion of Mattill and Golumbic that there is no difference between the muscular dystrophy of cod liver oil and that produced by lack of vitamin E   If this is true it is probable that the influence of vitamin E-free fat in the diet in protecting against or aggravating the onset of deficiency symptoms is entirely due to an anti-oxidant protection of the vitamin or a pro-oxidant aid in its destruction   Either might take place before or after the ingestion of the dietary mixture   A complete review of the studies on muscular dystrophy has just been published by Pappenheimer (150) in this journal

Vitamins of the B complex   The very striking sparing action that dietary fat has on thiamin has long been recognized   While the excellent reviews that have been written on this subject, especially those of Williams and Spies (194) and Cowgill (36), adequately bring the literature up to recent years, a few contributions should be discussed

Stern, Arnold and Elvehjem (170) in a study of the B_1 sparing action of fat, isocalorically replaced the carbohydrate of a B_1-deficient diet with fat and found that rats which had developed polyneuritis through previous subjection to a low fat, thiamin free regimen grew well, exhibited no signs of polyneuritis, and the females had normal estrous cycles   The symptoms of polyneuritis which the rats had when the fat diet was started did not completely subside for about 1 week although growth during this time was good. These authors point out

that since thiamin administration causes the disappearance of polyneuritic symptoms within 4 to 6 hours, fats must act in a manner independent of thiamin Salmon and Goodman (164) had previously reported that a diet containing 40 per cent fat, but very low in thiamin, did not prevent the occurrence of poly-neuritis in rats   Stern, Arnold and Elvehjem offer the suggestion that perhaps the diet used by Salmon and Goodman was low in some dietary factors other than thiamin for when the autoclaved yeast content of a high fat diet was in-creased symptoms of polyneuritis disappeared   It has been well established by the use of autoclaved and synthetic fats that the sparing action is not due to the presence of thiamin   Melnick and Field (141) have carried out chemical determinations for thiamin in fats, with negative results   Furthermore, Kem-merer and Steenbock (102) found no increase in the amount of thiamin in body tissues when thiamin deficient rats were fed high fat diets, and Cahill (32) has found that in humans ingesting a diet with a constant thiamin content, the addi-tion of fat had no influence on the amount of thiamin excreted in the urine These results together with recent information on the participation of thiamin in the carboxylase system indicate that fat spares thiamin by altering metabolism in such a way as to circumvent its need   An observation that could be inter-preted as a confirmation of this idea has been presented by Banerji (6) who found that the ingestion of a high fat diet not only alleviated the severity of a thiamin deficiency bradycardia, but diminished the excretion of bisulfite-binding sub-stances in the urine

Evans, Lepkovsky and Murphy (65) noticed an apparent aggravating effect of fat upon the vitamin G requirment of rats   More recently Mannering, Lipton and Elvehjem (127) have shown that fat at a level of 25 and 40 per cent of the diet, markedly increased the riboflavin requirement of the rat, while with the dog the level of dietary fat has no influence on the need for this vitamin (152)

Dietary fat seems to have some sparing action on the pantothenic acid (188) and biotin (147) needs of the rat   Furthermore certain fats (corn oil) give a much greater protection from egg white disease than others (Crisco), an observa-tion that might be associated with their content of the essential fatty acids (120)

Rancid fat   In the foregoing discussion some mention has been made of the deleterious effects of oxidized fat in the diet, particularly in the destruction of vitamins A and E   Serious as the effect on these two vitamins is, the detri-mental properties of rancid fat are much more extensive

The stability of all the essential nutrients to oxidized fat is not known, but on the basis of chemical structure and properties, the following substances might be considered capable of destruction by such pro-oxidants   Vitamin A, carotene, vitamin D, vitamin E, pantothenic acid, pyridoxine, biotin, ascorbic acid, and linoleic acid   There is a serious lack of information concerning the possible destruction of many of these substances by oxidized fat, but that information which is available will be discussed here

It was pointed out by Mattill and Golumbic (130) that many investigators working with E deficient animals have occasionally encountered dermatitis in

older animals and caudal necrosis in the new born   The dermatitis is sometimes relieved by the administration of wheat germ oil   As the wheat germ oil *per se* is possibly not responsible for the cure of dermatitis symptoms it may have protected other factors by its antioxidant properties   Neither pantothenic acid nor pyridoxine have been studied, but at least one of the dermatitis preventing vitamins, biotin, has been shown to be destroyed by organic peroxides (23) and by products produced in rancid fats (151a)

While the most important factor concerned in the oxidation of vitamin D in animal foods seems to be the amount of exposed surface area, rancid fats are also of importance in its destruction (71)

Ascorbic acid, which is known to act as a fat antioxidant, is not appreciably destroyed during the induction period (74)   Experiments from the authors' laboratory indicate that when pure ascorbic acid is suspended in highly rancidified fat it is not oxidized

The peroxidation of unsaturated fats naturally destroys linoleic acid   Feeding of such oxidized fats has been shown to result in the development of skin changes in rats (192) and dogs (191) suggestive of linoleic acid deficiency, but in a much more severe form than that ordinarily produced by exclusion of the essential fatty acids

The presence of rancid fat in the diet has effects other than in destroying essential nutrients   György *et al* (78) studied a diet of the following composition Casein 6, cornstarch 50, sucrose 22, cod liver oil 2, salts 4, and linoleic acid (crude) 16 with daily supplements of thiamin, riboflavin, pyridoxine, and pantothenic acid   Within 3 to 4 weeks standing at room temperature the linoleic acid was almost completely destroyed by oxidation   If butter yellow (N N -di methylaminoazobenzene) was incorporated in the diet at a level of 0 06 per cent, it was destroyed by the oxidizing linoleic acid   These authors are of the opinion that such substances as Crisco, butterfat, or rice which have a pro carcinogenic effect when given together with butter yellow do so by stabilizing the carcinogen either by reducing the unsaturated acid content of the diet or through an antioxidant effect   However, this can not be the explanation for the enhancing effect of dietary fat on tumor production by skin application of 20 methylcholanthrene for in this case rancidified fat is equally effective (110) Of striking interest in the study by György *et al* was the observation that the diet containing linoleic acid was very toxic   Rats fed this regimen either with or without butter yellow, lost weight, developed an anemia of the secondary type, a leucopenia, and were subject to pediculosis   These symptoms could be prevented by the daily addition of yeast to the diet   The toxicity was apparently due to oxidation products of the unsaturated fatty acid

In this laboratory loss of weight and death have been noted when a balanced diet containing casein, glucose, lard, salt mixture, and pure B vitamins together with cod liver oil and wheat germ oil was fed to rats   The toxicity of this diet was related to the development of rancidity, but as cod liver oil has been included in the diet in the same manner as in the György study it was thought probable that a deficiency of vitamin A had developed   Yeast was also found

protective, but on further study it was found that the yeast exerted a strong antioxidant effect which protected the lard from oxidation   When cod liver oil was fed separately and oxidation of the fat was prevented by refrigeration no toxic symptoms were manifested, but if the lard was rancidified to a peroxide value of 200 to 400 milli-equivalents per 1000 grams and again the cod liver oil fed separately loss of weight, decreased hemoglobin, and red and white blood cell count resulted   The livers of rats showing these symptoms had normal amounts of vitamin A, so the toxicity must have been due to other causes

The anemia that has been reported by György *et al* and also observed in this laboratory might be due to some indirect cause   It is of interest that Haurowitz, Schwerin, and Yenson (83) have found that in the well known catalytic effect of hemin and hemoglobin on the oxidation of unsaturated fatty acids, both pigments are completely destroyed   In this reaction inorganic iron is released, but neither porphyrins nor bile pigment derivatives are found   If there is any physiological significance in these observations, the oxidation products of the unsaturated acids that act as pro-oxidants must be absorbed from the intestinal tract and enter the blood stream   While there is at present no definite answer to this point there are reports that have some bearing   Irwin *et al* (99) report that hydrogenated cottonseed oils having peroxide values of 107 6 and 80 1 and melting points of 45 3° and 45 6°C respectively are absorbed to the extent of 90–92 per cent within 12 hours   Unpublished results from this laboratory show that when highly oxidized fat is ingested by rats the keeping time of the animals body fat is decreased   This does not necessarily mean that pro-oxidants are deposited in the adipose tissues for the peroxide value of the freshly rendered body fat is zero   Furthermore as was mentioned above the vitamin A stores in the liver are maintained at a normal level   The decreased keeping time of adipose fat after the ingestion of rancid fat could be due to the complete destruction of all dietary antioxidants   Under normal conditions these antioxidants might be absorbed and help stabilize the body fat   It is very likely that some products of oxidation are absorbed from the intestinal tract   For example, heptyl aldehyde (35) which is known to occur in small amounts in rancid fat, is readily absorbed and enters the body tissues   If harmful products of rancidification are absorbed they must do so to only a small extent for parenterally administered these substances exhibit a toxicity far beyond that observed following oral ingestion (48)

From the foregoing discussion it can be seen that the presence of highly oxidized fats accelerates the destruction of a large variety of food factors   While most of the detrimental effects of ingestion are probably due to induced nutritional deficiencies the possibility remains that some activity is directly manifested in the body tissues   The inadvertent addition of oxidized fat to a diet mixture, the development of fat oxidation in the diet due to lack of proper stabilization and the possibility of oxidative changes in the intestinal tract must be given serious consideration in practical nutrition as well as in the theoretical interpretations of experimental studies

The value of dietary fat as measured by growth and reproductive performance Many experiments have demonstrated that growth, lactation, reproductive

performance and other metabolic functions are altered by the consumption of fats. The results obtained are not always readily correlated with the composition of the fats and their expected digestibility, mobilization and retention, essential fatty acid content or effects on other nutrients. From the preceding discussions it is conceivable that fats of widely different fatty acid contents might have very different nutritive values. The cases to be cited below may be considered typical of the scores in the literature which record effects of quantity or quality of dietary fats

Nearly all experiments have shown that the inclusion of fresh, palatable and digestible natural fats improves the diet in some way. Infants on fat free diets develop loose, mushy, fermentative stools (96). Calves do not grow as well on skim milk supplemented with fat soluble vitamins as on whole milk (77) or re constituted milk into which butter oil, lard or beef tallow had been homogenized. On low fat the average daily gain was 1 07 pounds as compared with 1 43 pounds for the whole milk group. Cows (131) (72) and goats (12) produce less milk and butter fat on low fat diets. On a ration containing only 0 45 per cent ether extract decreases in milk yield of goats ranged from 25 to 55 per cent and the butter fat yield dropped 35 to 70 per cent. With cows the effects have not been so striking but a summary of experiments done by Maynard and co-workers over a period of 12 years has demonstrated that diets containing about 6 or 7 per cent of fat increase yields of both milk and butter fat as compared with diets containing less than 4 per cent fat. The depression of milk and butter fat yields, very marked when cod liver oil or menhaden oil is fed to cows (134) (22), is slight or lacking on the inclusion of shark liver oil (134) (161) (43) salmon oil (134), or unsaturated plant oils like corn oil (183) in the diet. In fact some of the above increases were obtained with the highly unsaturated soybean oil. Growing chicks (162) have been kept 14 weeks on a grain ration so low in fat that the iodine number of the body fat was greatly reduced without retarding the growth significantly although the average weight of both males and females on the low-fat ration was already slightly low at the end of this short (14 week) period. When laying hens were put on a diet containing less than 0 1 per cent fat there was a tendency toward a lower and less sustained egg production (163)

Many experiments with rats are not useful to this discussion because proper low fat diets were not used as controls or growth and reproductive performance was not recorded. However, there are a number of experiments which show definite improvement on the inclusion of some fat in the diet (in excess of the essential fatty acid requirement). Diets containing only the fat in crude casein and yeast and supplemented by 2 or 3 drops of cod liver oil do not produce maximum growth ovulation or lactation (61) (62) (63) (64). Twenty and twenty five per cent of lard, butter, or cottonseed oil is better than five or ten per cent of lard. It is not certain that the low fat diets were always adequate in the essential fatty acids. With higher levels of fat it has been found that diets containing 30 to 55 per cent of different fats produce greater weight gains in young rats than does a 5 per cent diet (91). In this laboratory at the present time there are groups of rats on diets containing 20 per cent of several fats. All are superior in growth and lactation to controls receiving 10 drops of corn oil (Mazola) daily. The

average weight of the high fat animals is 13 per cent above that of the low fat group   It is not known in any of these cases whether the increased weight is due entirely to obesity   However, with mixed diets of natural foods containing 4 50 and 9 29 per cent of fat, it has been recently shown that rat mothers with the higher fat intake show improved lactation and that the young at 17 days of age contain more dry matter, more fat and more protein (132)   There are, however, *experiments in which rats receiving up to 55 per cent of their diet as fat did not* grow more in the first six weeks than the controls on sucrose (164)   There is also the experience of those working with fat deficiency that fat-free diets supplemented daily with only 25–100 mgm of linoleic acid produce fair growth and lactation (124) (156)   In the first case (124) the performance is definitely below optimum and in the second (156), while it is said to compare favorably with that of the stock colony, no exact comparison of weaning weights is given   There is a preponderance of evidence that several species respond favorably to added dietary fat   Since all of these species have great powers of fat synthesis from carbohydrate (except for certain unsaturated acids) and are capable of utilizing either of these food materials for work with almost equal efficiency (107) it is not surprising that the differences are small   Nevertheless for optimal nutrition fat should furnish a considerable proportion of the calories

Large differences have been reported in the comparative nutritive value of different lipids, both natural and synthetic   The outstanding feature of these experiments is the irregularity of results from different laboratories and even within one laboratory   Most of the work has been done with rats on a basal diet to which have been added equal amounts of the fats under test   Usually ad libitum feeding has been allowed and the overall growth effects recorded   In *some cases, however, the gain per calorie intake has been calculated* or the food consumption limited and the gain per unit of fat recorded   Since there is little difference in the composition of different batches of commercial fats, it seems likely that the method of feeding or unrecognized effects of rancidity on the rest of the diet may account for the lack of uniformity   Lacking space for a detailed discussion here of each experiment with comments on possible factors affecting the results, only a few of the older experiments will be given along with the most recent ones

From the work of Ozaki (149), Eckstein (51) (52), Powell (154) (153), Davis (42), Barbour (8), Evans and Lepkovsky (62) (63) (64) (113), Cox (37), and Salmon and Goodman (164) with synthetic esters and salts of the fatty acids it is evident that the comparative growth promoting value of these materials depends upon the form in which they are fed, the percentage in the diet, and the adequacy of the rest of the diet   Even as much as 60 per cent of the diet may be composed of the methyl, ethyl, glycerol or propylene glycol ester of mixed fatty acids of lard without ill effects (113), only the ethylene glycol ester being toxic   Soaps may also be fed successfully at lower levels   In general the glyceryl esters seem best   This is especially true when single fatty acids make up as much as 75 or 80 per cent of the calories   For example, ethyl laurate is very toxic at this level (37) while laurin is tolerated and promotes growth (164)   Butyrin and

ethyl butyrate are highly toxic as contrasted with the esters of acetic or caproic acids which allow fairly good growth   There is a sharp distinction between the 4-carbon acid and its neighboring homologues   Its ill effect on growth is due not only to a depression of appetite resulting in starvation but to an injurious effect upon tissues (42)   Toxic effects of the other single fatty acids become evident only when they are fed at very high levels and the admixture of other acids eliminates the difficulty   In practically all cases the natural fats are superior to glycerides resynthesized from single or mixed fatty acids   In fact, only when the best of natural fats are added is the performance better than on a low fat-high carbohydrate diet   The addition of 20 per cent or more of synthetic esters of single or mixed fatty acids usually results in growth inferior to that on the basal diet (64) (164)

The order of relative efficiency of different fats may be reversed by a change of level in the diet as well as by a partial deficiency in the diet such as thiamin (64) (164), or vitamin A (24)   Ad libitum feeding may reverse the order as compared with restricted feeding

There are many experiments which demonstrate that, with not over 30 per cent of fat in the diet, the growth of rats is good on fats ranging in composition from coconut oil to linseed oil (25) (91)   When very great differences due to small amounts of fat are observed the diet should be examined carefully for some deficiency, possibly due to the action of the fat itself   The effects of different oils are not shown as strikingly with rats as with calves which fail to grow, decline, and die when liquid fats are homogenized into skim milk (77)   But with the rat there is considerable evidence that some liquid fats like cottonseed or corn oil do not equal solid fats in promoting early growth (164) (25) (91), especially when they constitute a large part of the diet   Although the superiority of butter fat for calf nutrition seems established it is not so readily demonstrated with the rat Using the method of homogenizing fats into mineralized skim milk more rapid early growth on butter fat has been found (18) (24) but the results are sometimes small and not always regular   These differences in early growth were not found by Freeman and Ivy (69) when commercial evaporated milk was compared with a filled milk containing coconut oil instead of butterfat   After three months, however, the animals receiving butterfat were larger than their controls With simplified diets it has been found by Harris and Mosher (82) that the results can be reversed by using animals of different age, weanling rats growing more rapidly on butterfat and older rats more rapidly on coconut oil   Premature babies and twins, in whom fat utilization is defective, absorb olive oil and soy bean oil far more completely than butter, with a resulting improvement in growth and nutritive condition (185)   Some early experiments with solid diets indicate a superior value of butter for lactation (61), but more recently Sure (182) has reported no differences in lactation performance on diets containing 15 per cent of several fats, including butter   Recently Boer and Jansen (16) have postulated a new growth promoting fatty acid in butter   However, Euler, Euler and Säberg (58) report better growth of rats on margarine   In our laboratory at the present time there are six groups of rats which show that at the 20 per cent level

butter is just equal to several other fats   Some older experiments, in which very high levels of fats were used (55 and 60 per cent of the diet), show distinctly superior growth on other fats as compared with butter (164) (62)   If each of the other commercial fats is reviewed, conflicts in results will be found similar to those mentioned above for butter   The answer seems to lie in the variability of the products used by the workers as well as in the differences in methods of experimentation   The former effect is well illustrated by the recent paper of Hoagland and Snider (92) in which several lards are compared with several hardened vegetable and animal shortenings   The average growth rate of rats for all the lards was essentially the same as for all the shortenings   However, the lards differ significantly among themselves   Until all workers use exactly the same materials and conduct the experiments under identical conditions, conflicting results will continue to appear   From the explanation of these differences will come a more complete knowledge of the rôle of fat in the diet

SUMMARY

There are ample reasons for recommending that the fat intake be not reduced much below the normal established by habit   To give the best results added fats must be fresh   Rancidity renders them unpalatable, destructive to other vital foods and possibly slightly toxic in themselves   Frequently the preservation of a mixed food is largely a matter of the prevention of fat deterioration

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QUANTITATIVE AND QUALITATIVE VARIATIONS IN NORMAL LEUKOCYTES

CYRUS C STURGIS AND FRANK H BETHELL

Thomas Henry Simpson Memorial Institute for Medical Research, University of Michigan

Two excellent reviews have been written dealing primarily with changes of the leukocytes in the circulating blood under different physiological conditions, in man by Garrey and Bryan (1936), and in rabbits by Cheng (1930)   The reader is referred to these publications for a comprehensive consideration of the litera ture and summary of the status of our knowledge concerning the subject to the date of each publication   The present article was requested because some im portant contributions have been made since the appearance of the two reviews mentioned above   The material in recent articles has been based on studies which, in some instances were more accurate technically, due to the use of standardized apparatus and technic, the results have been treated by modern statistical methods, more important observations have been made on changes in the differential formula and on the maturity of the neutrophils as indicated by the shape of the nucleus   Furthermore there has been a growing appreciation that under normal conditions, at short intervals, there is a spontaneous and irregular fluctuation of the total number of leukocytes and of the percentages of the different varieties of white corpuscles, which may easily be misinterpreted unless evaluated critically   Hence any changes in the leukocytes, especially when they are within the range of normal, or moderate in extent, should not be interpreted as due necessarily to any single or constant physiological or patho logical influence, unless the trend is a uniform one in multiple experiments

Recognition of the cause and extent of the physiological variations in the leukocytes is important when fluctuations in their number and variety are utilized as evidence of significant alterations either in experimental or human physiology   Undoubtedly conclusions have been reached without proper allow ance for observational error spontaneous variations, or the uncertainty of isolated determinations.   In clinical medicine, decisions have been based on one or a few widely spaced leukocyte counts, with a differential formula determined by a survey of only one hundred cells and with a total disregard for their physio logical variations   Since the introduction of the sulfonamide drugs, for example, there has been a tendency to regard a decrease in the white blood cells as evi dence of the toxicity of the drug and hence as an indication for its discontinuance, even though the diminished total white count remains within normal limits   As a consequence, an erroneous interpretation may lead to the denial to the pa tient of an exceedingly valuable and sometimes life saving therapeutic agent

Our present knowledge indicates that variations in the white blood cell count of the circulating blood may occur as the result of the action of any one, or a combination, of the four following mechanisms

I Alterations in the rate of production of leukocytes   A decrease in the leuko-

exclusion of such factors as thickness, evenness, and length of film, and permitted the inference that the observed variations were due to irregularities of cell distribution, especially along the edges of the films   The differences observed between drops were also greater than might have been anticipated as due to random subsampling   Therefore, more accurate results may be secured by counting on films prepared from two drops of blood, than by utilizing only one drop, even though the total number of leukocytes enumerated is the same in both cases

Although Mainland and his associates point out that the distribution of blood corpuscles, as they are counted on a film, is discontinuous and therefore not truly represented by a normal curve, they submit data justifying the assumption that for purposes of statistical calculation the distribution may be considered as symmetrical when the conditions of examination are strictly defined   On this assumption, for the 24 healthy male students, aged 19 to 28 years, whose bloods were examined in the early spring, the following figures are submitted based on a count of 2100 leukocytes for each student (table 1)

TABLE 1

	MEAN PERCENTAGE	STANDARD ERROR OF MEAN	STANDARD DEVIATION OF SERIES	COEFFICIENT OF VARIATION
Neutrophils	56 88	±1 201	±5 886	10 4
Eosinophils	2 01	±0 209	±1 023	50 9
Basophils	0 44	±0 042	±0 204	46 5
Lymphocytes	37 03	±1 221	±5 982	16 2
Monocytes	3 64	±0 168	±0 825	22 7

The unavoidable error in differential counting, or that due to the accidents of random sampling where the distribution is assumed to be normal, has been calculated by Barnett (1933) according to Bernoulli's theorem $SD = \sqrt{n\,p\,q}$, where SD is the standard deviation, n is the total number of cells counted, p is that fraction of the total made up of the particular cell type in question, and q is that fraction of the total composed of all the remaining cells   Barnett checked the application of this theorem to the differential count experimentally by a series of counts of 100 cells each, made on cover slip preparations from the same sample of venous blood, and found reasonably close agreement between the observed and predicted cell values   He has constructed a chart showing maximum errors due only to chance in differential counts based on the enumeration of 100, 200 and 400 cells   The maximum error he defined as three times the standard deviation   Thus if 100 cells are counted and 50 per cent are neutrophils the standard deviation is 5 per cent and the maximum error is 15 per cent   When the total number of cells is 400 instead of 100 the respective values are halved   Barnett concludes that at least 400 cells should be counted in order to obtain reliable results in the differential count

The validity of the above theorem, as applied to differential counts, was also tested by Plum (1936), who counted on a number of slide films from 50 to 100 sets of cells, each set being composed of from 50 to 100 members   He examined

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Recognition of the cause and extent of the physiological variations in the leukocytes is important when fluctuations in their number and variety are utilized as evidence of significant alterations either in experimental or human physiology   Undoubtedly conclusions have been reached without proper allowance for observational error, spontaneous variations, or the uncertainty of isolated determinations. In clinical medicine, decisions have been based on one or a few widely spaced leukocyte counts, with a differential formula determined by a survey of only one hundred cells and with a total disregard for their physiological variations   Since the introduction of the sulfonamide drugs, for example, there has been a tendency to regard a decrease in the white blood cells as evidence of the toxicity of the drug and hence as an indication for its discontinuance, even though the diminished total white count remains within normal limits. As a consequence, an erroneous interpretation may lead to the denial to the patient of an exceedingly valuable and sometimes life-saving therapeutic agent

Our present knowledge indicates that variations in the white blood cell count of the circulating blood may occur as the result of the action of any one or a combination, of the four following mechanisms

I Alterations in the rate of production of leukocytes   A decrease in the leuko-

cyte count may result from simple inhibition or maturation-arrest in the bone marrow, whereas an increase is dependent upon simple hyperplasia or accelerated maturation

II ` Variations which are dependent upon changes in their rate of destruction or elimination from the body

III Changes in the number per cubic millimeter resulting from differences in concentration of the circulating blood plasma

IV Redistribution of the white blood cells in the vascular channels   Evidence in recent years tends to reaffirm the earlier belief that this mechanism is the basis for many, if not all, of the physiological variations in the leukocyte count

The above classification is based, with modifications, on the information presented by Lawrence (1941), to which reference should be made for fuller details ,

OBSERVATIONAL VARIATIONS   Variations in total leukocyte counts attributable to observational error may be separated a priori into two classes 1, the "error" involved in drawing conclusions relative to an entire population on the basis of data derived from observations on small random samples, 2, "blunders" introduced by defective technical methods and inaccurate apparatus   Although, in practice, one cannot disregard the influence of "blunders" in the evaluation of individual counts, it is nevertheless possible so to standardize the conditions of observation that variations introduced by the second class become negligible thereby permitting a statistical approach to the "error" of random sampling Bryan, Chastain and Garrey (1935) point out that this error is influenced by three factors, a, mixing of the cells and diluting fluid, b, filling the counting chamber by capillarity, and c, settling of cells by chance on the ruled field of the counting chamber   They are able to demonstrate that the last named, chance settling of the cells, is responsible for practically all of the major variations of subsampling   It therefore becomes possible, within reasonably accurate limits, to apply the laws of a normal frequency distribution system in the statistical evaluation of total leukocyte counts, when the conditions of observation are sufficiently well standardized   These authors also point out that more accurate expression of the "error" of physiologically normal leukocyte counts is gained by the use of a scale of absolute numbers rather than percentages since the latter are partly dependent on the means of the leukocyte counts   Based on an analysis of 2508 white blood cell counts they report an average error of ± 241 ± 35 cells per cubic millimeter when 10 unit hemacytometer areas of one square millimeter each and a blood dilution of 1 to 20 are employed   The magnitude of this error did not alter appreciably at the different levels of the observed leukocyte counts as was shown by the low value of its probable error

It has also been demonstrated by Plum (1936) that when the conditions of leukocyte enumeration are adequately controlled the accidental distribution of cells in the counting chamber becomes the only important variable   His data, obtained by empirical observation, led him to conclude that a systematic relationship exists between the magnitude of the standard deviation and the number of

cells enumerated in each determination, and that this relationship may be ex
pressed in terms of a Poisson's series according to which the standard deviation
is equal to the square root of the counted number of cells

For a laboratory procedure in such common use as the determination of
percentages of the several types of leukocytes, the differential count, the many
technical methods employed and the lack of exact information concerning their
respective accuracy constitute a striking deficiency in hematologic practice
Moreover, few observers possess well founded knowledge of the statistical error
involved in the differential count when the distribution of the cells in the stained
film is assumed to be entirely "accidental '

Differential counts of five entire blood films made on slides with a cover slip
spreader, and an almost complete count on a sixth, were compared by Mainland,
Du Biher and Stewart (1935) with four clinical methods of differential counting
It was found that the results obtained either by counting three sets of 100 leuko
cytes, using cross rows, one 3 mm from the beginning, the second at about the
middle and the third about 3 mm from the end of the film, or by counting all
the cells in the middle longitudinal row one field in width from beginning to end
of the film, agreed sufficiently closely with the total counts to be attributed to
random sampling   On the other hand more complicated procedures, such as
Schilling's "meander four field ' method and Silberstein's method, showed dis
crepancies greater than are usually attributable to chance   For the cross
count method, with allowance for the size of the samples employed the authors
estimate a range for the neutrophil percentage, attributable to random sampling,
from 7 4 per cent above the true value to 7 4 per cent below it   The minimum
ranges of error for the other cell types were lymphocytes, 6 1 per cent, mono-
cytes, 2 6 per cent, eosinophils 2 4 per cent basophils, 1 0 per cent. These
values, derived from small samples for which due allowance was made, should
include about 99 per cent of all observations   In a later publication, Mainland,
Coady and Joseph (1935) estimate the observational variation for the cross count
method of differential enumeration using slide preparations   They point out
two sources of variation affecting the differential count, apart from the error
involved in truly random sampling   These variations are well recognized, but
frequently overlooked by the laboratory worker   They consist of 1, abnor
malities of distribution of the several types of leukocytes on the film, as prepared
by the slide method, so that in differential counting one is ' taking a sample
merely of the area of film examined not a representative sample of the whole
drop", 2, differences between successive drops of blood and between blood ob-
tained from more than one puncture   Their data were obtained from twenty
four healthy male students   Across each of seven blood films, prepared by the
slide and cover glass method, five from successive drops from a right ear puncture
and two from successive drops from a left ear puncture, made on the same oc
casion from each student, three sets of 100 leukocytes each were counted   The
variations of these sets were greater than could be attributed to purely random
sampling, indicating, therefore, that in the comparison of differential counts the
methods employed must be strictly adhered to   Their technique permitted

exclusion of such factors as thickness, evenness, and length of film, and permitted the inference that the observed variations were due to irregularities of cell distribution, especially along the edges of the films   The differences observed between drops were also greater than might have been anticipated as due to random subsampling   Therefore, more accurate results may be secured by counting on films prepared from two drops of blood, than by utilizing only one drop, even though the total number of leukocytes enumerated is the same in both cases

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The validity of the above theorem, as applied to differential counts, was also tested by Plum (1936), who counted on a number of slide films from 50 to 100 sets of cells, each set being composed of from 50 to 100 members   He examined

consecutive adjacent longitudinal rows, starting at the margin and 'proximal' end of the film. The results showed that, in these experiments, the individual counts were distributed about the mean in the manner of the normal curve.

The normal frequency or Gaussian curve is not strictly representative of the distribution of most cell values in the differential count even though the "sampling error" is assumed to be the only source of variation, according to Goldner and Mann (1938). However, by a mathematical analysis, they demonstrated that the error involved in the application of the normal curve to all counts is negligible. Based on this approximation they have constructed "confidence curves" for differential counts of 200 leukocytes employing a "probability" of 95 per cent ("Fiducial Coefficient" of 0 95). The validity of the curves was tested by serial observations of a single sample of blood. For leukocyte percentages ranging between 30 and 70 the calculated "maximum" variation is approximately ± 6 per cent.

The several sources of possible error in the performance of the differential leukocyte count have been considered by MacGregor, Richards and Loh (1940) with particular reference to variations dependent upon technique of making the film and method of selection of areas for counting. On all slide preparations lack of uniformity of distribution of cell types was found to be the rule. Variations, in some cases exceeding 20 per cent were attributable to this factor. A general relationship was observed between the warb distribution pattern of one type of cell and that of any other type. In order to determine this relationship consecutive areas throughout slide films were counted and the values obtained subjected to Fourier analysis. From the data secured in this manner graphs were constructed. These graphs enable correlations to be made between the count determined by examination of all cells in the film and the results obtained by 1, edge" count along the two longitudinal margins of the film for a width of one microscopic field, 2, 'battlement' count three horizontal edge fields followed by two fields toward the center, then two horizontal and then two in a vertical direction back to the edge 3, 'cross-sectional" count, back and forth across the body of the film from one edge to the other until at least 300 cells have been counted. It therefore becomes possible, according to the authors to compare results obtained by different methods of counting by the application of correction factors varying with the type of cell and its observed percentage value. The accuracy of such 'corrections' is dependent upon a standardized technique of film preparation and is lessened in cases of total leukocyte counts differing widely from the normal. MacGregor and his associates found that closest approximation to the values obtained by a count of the entire film was provided by the battlement method utilizing both edges of the film. Greatest corrections were necessary in the case of counts made by the cross-sectional method, with the straight edge method occupying an intermediate position. Counts performed on cover slip films correspond more closely to the straight edge count than to either of the other two methods of counting on slide preparations.

The percentage of lymphocytes in the case of a healthy individual was found by Boveri (1930) to average about 10 per cent higher when determined on slide

films by the cross count technique described above, than when enumerated in cover slip preparations or in a Buerker counting chamber   The counting chamber is assumed by the author to give most nearly perfect random distribution On the basis of removal of four-tenths of the granulocytes and monocytes from the countable portions of a slide film the author has constructed a curve showing the corrections that should be made in order to obtain the true lymphocyte percentages from values obtained by counting slide preparations

The four-field meander method, counting from one side of the film to the other and back, and differential enumeration of cells in a counting chamber were compared by Klotz (1939) for constancy of results when single samples of blood were used   The slide films were made in a uniform manner, standardizing the size of the drop, the angle of the spreading slide and the pressure exerted when making the spread   Random cross counting gave results considered to be more accurate than either the four-field or hemacytometer chamber methods

From the rather widely differing conclusions of the several authors whose work has been described above, it is apparent that no agreement has been reached regarding either the preferred method of differential counting on slide preparations, or the degree to which distribution of cells approaches the wholly accidental on either slide or cover slip films   However, it has been the experience of most observers who have compared the slide and cover slip methods, that slide films show a distinct predominance of neutrophils and monocytes at the edges and tail of the preparation, and that even with a well standardized technique, this inequality of distribution varies with the concentration of the red cells and the number of leukocytes in the blood, whereas, in cover slip films, utilizing only the weight of the upper slip to effect the spread, the distribution of white cells is more nearly uniform   It is obvious that the method employed in selection of areas for differential counting becomes less important as the distribution of cells approaches the "accidental "

√ DAILY AND HOURLY VARIATIONS   It has long been known that fluctuations in the leukocyte count may occur throughout the day, but originally these were attributed to exercise or digestive activity   Later it was recognized that they arise independently of these possible excitants   In 1925 Sabin, Cunningham, Doan and Kindwall reported that a characteristic hourly rhythm of the total white blood cell count occurred in man and rabbits, and that this accounted for a variation in a proportion of 1 2   Furthermore, they found that the white blood cell count was increased in the afternoon, independent of food intake, and that the entire increase was due to changes in the number of neutrophils   They also concluded that the lymphocytes have a shorter rhythm than the neutrophils, and that their total number varies in the proportion of 1 3   The suggestion that oscillations characteristically occur at hourly intervals has not been confirmed   Casey, (1940), after a most extensive study, concluded that his studies on rabbits did not reveal "a statistically significant variation in the hourly mean "   Kennon, Shipp and Hetherington (1937) in an investigation of the leukocyte count in young men likewise failed to discover "proof of rhythmicity occurring at regular intervals of short duration, either for the total number of

leukocytes or the total numbers of any one type of cell " It is true, however, that variations of considerable magnitude in the white blood cell count, due to the changes in the numbers of granulocytes, do occur in the blood of rabbits and man   Furthermore, these alterations are not related to food intake or exercise   According to Garrey and Bryan (1936) if one substitutes the phrase *irregular and sometimes pronounced oscillations* for *rhythms*, the apparent disagreements in the literature are of minor importance   These observers conclude that when leukocyte counts are done in the morning, under basal conditions, a great majority of the white blood cell counts will fall within the range of 5,000 to 7,000 cells per cubic millimeter   According to them, it is firmly established that the minimum count is observed in the morning, under conditions of rest, and that it varies least under these conditions   Furthermore, they state that after an hour of recumbent rest in the afternoon, 90 per cent of all leukocyte counts fall within the limits of the morning basal counts

In the study of Kennon Shipp and Hetherington (1937) the white blood cell count in six young men was determined at intervals of 15 minutes throughout the day for 5 to 7 hours, on two successive days   They observed that the total number of leukocytes varied from count to count, due to changes in the granulocytes, but no evidence of a rhythm could be determined, the undulations in the curve had a tendency to adhere to the same pattern in a given subject on successive days, after a rest of one-half to one hour there was a fall in the leukocyte count to the basal range, there was no significant alteration in the total white blood cell count when an erect posture was assumed, the apparent spontaneous variations in the white blood cell count throughout the day were very slight usually the difference being between 100 and 400 cells between the highest and lowest counts, but in one instance the maximum variation was 900, and in another, 1800 cells, while there was no steady consistent rise in the white blood cell count in the afternoon, in all instances there was a definite post meridian peak between twelve noon and 3 00 p m   and although the subjects had breakfast or a light lunch during the days of the study, there was no evidence of a digestive leukocytosis

Casey (1940) undertook a comprehensive statistical evaluation of a large number of white blood cell counts in normal rabbits in order to determine if a recognizable diurnal cycle in the leukocyte series actually existed in this species   The counts had been made not oftener than three times a week in a given animal and usually only once a week   In all, 597 estimations on 190 of the 204 rabbits were made between 9 00 a m and 12 00 noon, and 366 determinations of 136 of the 204 rabbits between 12 00 noon and 5 00 p m   It was concluded that no single individual hourly mean differed significantly from any other, what variation was present they considered, could be accounted for on the basis of random sampling   The white blood cell count averaged 7,891 per cu mm for the morning, and 7 914 per cu mm for the afternoon period, a difference of 23± white blood cells which is not significant   This investigator also concluded that there was no evidence that a digestive leukocytosis occurs in the rabbit between the hours of 9 00 a m and 5 00 p m , as no statistically significant change was ob-

served in the group permitted free access to food and water   It is recognized by Casey that previous observers have reported that the lowest white blood cell counts in man occur early in the morning and the highest in the afternoon, despite control of the food intake, muscular effort and emotional stress   It is also pointed out that repeated painful punctures cause a successive rise in the leukocyte count (Garrey, 1929)   Furthermore, Casey is critical of most of the studies of the variations and rhythms in the white blood cells, as he considers that they are based on an inadequate number of estimations, and no statistical consideration of observational variation seems to have been undertaken   His study, he believes, is significant and conclusive for a number of reasons, among them being only a single examination was made on an animal on any one day, the material was analyzed by accepted biometric procedures, it represents by far the most extensive study of the sort, which has been made   Certainly, his results seem, in general, to contradict the belief that there is a uniform diurnal rise in the leukocyte count in rabbits   It cannot be said, however, from the material presented, that individual rabbits do not show a diurnal rhythm, or an increase in the leukocyte count in the afternoon hours

As a prelude to a study of the variation in the leukocytes in rabbits following the injections of various substances, Reifenstein, Ferguson and Weiskotten (1941) have made a most careful study of the spontaneous variations at hourly intervals in the total leukocyte count, the differential formula, and the maturity of neutrophils in normal rabbits   Throughout the experiments there was careful control of the health and handling of the animals, the environment, and the diet Adequate precautions were taken to ensure the accurate estimation of leukocytes as 16 unit areas of the chamber were counted which meant that 800 cells were actually enumerated, when the total leukocytes numbered 10,000 per cubic milli- meter   Eight leukocyte estimations were made at hourly intervals on the same day between approximately 9 00 a m and 4 00 p m   Three blood films were made at the time of each count, and 300 cells were enumerated in order to cal- culate the differential formula   A group of fourteen adult New Zealand white and mixed rabbits of both sexes, weighing approximately 2 5 to 3 2 kgm each, were studied during the period from May 24, 1939, to January 23, 1940

The results show that there is considerable spontaneous variation in the leuko- cyte counts of each rabbit   This is apparent when the average of all eight counts on each day for each rabbit are compared   The average for the series of the fourteen animals was 9,449 per cubic millimeter with maximum average varia- tion from 6,414 to 15,014 per cubic millimeter   Repeated studies on the same rabbits at approximately the same hours of the day, on different days, show that each animal seems to have its own characteristic leukocyte picture and varia- tions appear to be decreased in extent under such conditions   The observers advise that when hourly data dealing with the leukocytes are determined for experimental purposes, it appears wise to employ the same animal after its individual leukocyte behavior has been determined, and then utilize the same period of the day for counting   When average hourly estimations for all of the fourteen animals are considered, the total leukocyte values are fairly constant,

the average number of neutrophils increase and the average number of lympho-
cytes decrease during the day   For example, the average hourly counts per
cubic millimeter for the fourteen animals, from approximately 9 00 a.m to 4 00
p m were as follows 9,000, 9,221, 9,021, 9,914, 9,300, 9,800   On the other
hand, the total leukocyte count in the same rabbits may vary from 7,000
per cubic millimeter at 9 00 a m to 14,900 at 3 00 p.m   This is not true of all
of the animals for in some the count may have a remarkable constancy   A
comparison of the average morning neutrophil counts with those in the after
noon shows that almost invariably there is an increase in these cells during the
latter part of the day   This varies from approximately 200 to 2,000 cells per
cubic millimeter   Occasionally there is a decrease   With the tendency to an
increase in the neutrophils during the afternoon, there is usually a decrease of the
lymphocytes of approximately 200 to 3,500 cells per cubic millimeter   Here
again there may be an occasional increase   The authors conclude their discus
sion with a very appropriate and practical warning, as follows "These results
seem to indicate that when the hourly leukocyte picture of the rabbit is studied
under different experimental conditions, it would seem advisable to determine
the general normal leukocyte trends of each animal and then maintain the same
period of the day for counting at hourly intervals under experimental con
ditions, as well as establish similar control of other possible sources of error in
the determinations "

An additional study by Reifenstein and Hilfinger (1942) merits careful con
sideration for they investigated in rabbits for the first time the variation in the
maturity of polymorphonuclear neutrophilic leukocytes from hour to hour during
the day   Total and differential white blood cell counts were made at hourly
intervals at the same time of the day for six hours on twenty normal adult ani
mals   The cells were classified according to the following grouping

Group I   Cells with the nuclear material in one mass, round, oval, or indented
not more than one-half through its width

Group II Cells with the nuclear material not divided into segments but may
be lobed, spiral looped, rosette shaped or variously irregular

Group III   Cells with nuclear material showing segmentation into two
parts which are either entirely separate (as viewed in the film), or connected with
a narrow filament.

Group IV   Cells with the nuclear material showing segmentation (as in
group III) into more than two parts

According to this arrangement the first and second groups comprise the
division known as the non filamented and groups III and IV the filamented
neutrophils

These results are expressed in the weighted mean which has the advantage of
indicating the maturity of 100 neutrophils by a single figure   It is computed
as follows The number of cells in each of the four groups is multiplied by the
number of the group (I, II, III or IV) all results are added and the sum is divided
by the total number of cells counted   According to them, the weighted mean is
an exceedingly sensitive index of nuclear classification as a difference of one point

in the second decimal place means that one neutrophil has been moved to the group either below or above its original assignment

A consideration of the results shows that each animal on its particular day of counting appeared to have a rather characteristic weighted mean   The only other change was that some seemed to be "more to the left" than others   There did not seem to be a tendency for the mean to shift to the left or right during successive hourly counts   In other words, there was a strong tendency to maintain the initial average neutrophilic maturity during the six hour period of counting

The percentage of the non-filamented neutrophils (groups I and II) varied from 11 to 48, with a mean of 28 5, median of 29 and a mode of 29   Only two group I-neutrophils were observed in classifying approximately 14,000 neutrophils   One table is presented showing the neutrophilic maturity counts on the same animal at approximately the same time of day (9 45 a m) on different days during a 23 months period   These data emphasized that while the level of the neutrophilic weighted mean in a rabbit may vary slightly over a long period of time, the relative constancy of the mean, once it has been established for any particular daily period of study, is maintained during this interval   In other words, when a general trend in the neutrophils has been determined for each animal, there is every reason to expect that there will be very little shift to the "left" or "right" during the succeeding six hours   These results would indicate that the ratio of the appearance of younger neutrophils in the blood to the disappearance of the older neutrophils from the blood is balanced or constant   The scarcity of group I cells suggests that rarely are cells of the neutrophilic series discharged into the blood until they reach a stage which is more mature than the metamyelocyte stage   Their studies would suggest also that any variation in the neutrophils which does occur at short intervals is not due to an increase in the more immature cells of this series   It is likely, therefore, that such variations are on the basis of a redistribution of the cells of the body

Medlar (1929, 1936), after an exacting study on normal persons and on patients with tuberculosis, reached the following conclusions concerning the fluctuations in the white blood cell count   In his opinion neither the total leukocyte count nor the percentage of the different types of white corpuscles has an absolute level   A variation in the total of 50 per cent and in the differential count of 10 per cent may occur in normal persons when counts are made at five to ten minute intervals, over a period of an hour or two   Such variations may occur at irregular and unpredictable intervals   He cautions that a significant fluctuation must, therefore, be more than 50 per cent of the total and 8 per cent of the differential picture   Furthermore, to be pertinent, it is necessary that the changes occur uniformly in subsequent observations   He noted no significant effect of rest and random activity on the total and differential count   This led him to conclude that distinctive "rest" and "activity" levels of the leukocyte count do not exist if the activity is limited to mild exercise   He is also of the opinion that "complete relaxation, such as a night's sleep or two hours in bed, appears to bring about a more irregular distribution of the leukocytes, both in

the total and in the differential count, than is present during a state of normal activity "

THE EFFECT OF ACTIVITY ON THE LEUKOCYTE COUNT   It appears to be clearly established that the leukocytes increase in numbers in the circulating blood following muscular exertion   This is a constant finding and, furthermore, the leukocytosis is, to a certain extent, proportional to the duration and severity of the muscular contractions   Reviews of the literature bearing on this subject have been published by Grawitz (1911) and by Garrey and Bryan (1935)   Exercise of any type increases the leukocyte count, even "random activity" causing a moderate rise in the count   It has been established that the leukocyte level is lowest during periods of minimal activity (Bryan, Chastain and Garrey, 1935) and that the correct basal count is the one made in the early morning under conditions of rest (Naegeli, 1931)   Following the initial observations of Schultz (1893) it was shown that Marathon runners at the termination of a race uniformly had a leukocytosis which varied between approximately 14,000 and 27,000 cells per cubic millimeter, and a percentage of polymorphonuclear leukocytes which ranged from 80 to 90 per cent of all the white blood cells   It is generally believed that the increase is due to a redistribution of the white blood cells in the vascular system rather than to a formation of new leukocytes   This is indicated by the rapidity with which the increase may occur and by the fact that only a small percentage of the cells are of the younger types   It has been recorded immediately following a quarter mile race, lasting less than one minute, that there is a leukocytosis of 35,000 per cubic millimeter   Isaacs and Gordon (1924) report that at the termination of a Marathon race the polymorphonuclear leukocyte count percentage may be as high as 94 7   That this increase is attributable to a redistribution of cells in the vascular system is shown by the fact that in all cases examined by them the polymorphonuclear neutrophil leukocytes appeared to be of the mature, adult type, with clear-cut nuclear lobulation   It is generally believed that the changes in the circulating blood following exercise are due to circulatory shifts with the liberation of sequestered leukocytes from unused capillaries throughout the body   These reservoirs, in which the polymorphonuclear cells are stored, may be in various organs and tissues of the body such as the spleen, liver, lungs, glands of internal secretion, bone marrow, and muscles

That the degree of leukocytosis is due to the intensity and the duration of the exertion is shown conclusively by the observations of Edwards and Wood (1932) who noted an increase of nearly 300 per cent in the white blood cell counts of football players although the average duration of actual play in a sixty minute game is only eight minutes   This degree of leukocytosis is surpassed only by that observed in Marathon runners   Furthermore, it was found that the degree of leukocytosis was directly proportional to the extent of their participation in the game, as shown by the following figures   after playing one-quarter, the average count was 12,000 per cubic millimeter, one half, 15,500, three-quarters, 18,000, and the entire game 23,000

It is claimed by some that the emotions of fear, rage and apprehension may

raise the count to activity level (Garrey, 1929) but Edwards and Wood (19: consider that excitement alone is without effect upon the leukocyte cou This they demonstrated by determining the leukocyte count on spectators games, football players immediately before games, and track athletes just pr to a race   Leslie and Zwemer (1935) failed to obtain an increased leukoc; count in cats with excitement alone and therefore concluded that they wi unable to show any increase in the white blood cell count from emotional stim lus in the absence of activity   For a further discussion of this aspect of t subject see under "Emotion "

It is claimed that lactic acid of the blood, blood sugar, blood pressure, bo temperature and capillary dilatation can be ruled out as separate causes-relat to leukocytosis in exercise (Edwards and Wood, 1932)   The leukocytosis exercise seems to be proportional to the extent of the alterations in the circu tory rate of the body   As this is accelerated, the sequestered cells are swe into the circulation causing a physiological leukocytosis   Furthermore, th remain there for a considerable period of time before, as it is reasonable to i sume, they gradually settle out again in the various capillaries of the body wh the circulation becomes slowed   Leslie and Zwemer (1935) believe that t main factor in the prompt production of leukocytosis following exertion is t dilatation of collapsed capillaries in the muscles of the body   They state th during rest there are vast numbers of leukocytes adherent to inactive vess walls   These become detached when the capillaries open during exercise, a swept into the circulation, and account for the rise in leukocytes   The pc sibility that the spleen may harbor a reserve store of leukocytes which enter tl general circulation when this organ contracts following adrenalin injection thereby causing a transient leukocytosis, has led some to emphasize that th viscus may play an important rôle in the production of physiological elevatic of the white cell count   This is undoubtedly true but it has been demonstrate that the injection of adrenalin will cause such a leukocytosis in persons followir splenectomy (Lucia, Leonard and Falconcer, 1937)   A more likely explan; tion of the increase in number of leukocytes is that the adrenalin increases th rate of blood flow and thereby redistributes the formed elements of the blooc Thus it is not difficult to understand why there is a leukocytosis in exercise (any type, or, in fact, in any condition in which the circulatory rate is increase(This may occur in random activity, in the labor of a pregnant woman, in athlet contests, following the injection* of adrenalin, in excitement, and followir convulsive seizures

It should be pointed out that in some persons the leukocytosis is due to a increase in lymphocytes instead of polymorphonuclear cells   For examp' (Garrey and Bryan, 1935), a high grade of leukocytosis may show a preponde ance of lymphocytes   It is also reported (Jokl, 1931) that the blood pictui undergoes three changes following exercise first, an increase in the percentag of lymphocytes following strenuous exercise such as a race of 2,000 meter second, a preponderance of polymorphonuclear leukocytes after a race of lon duration (10 to 25 kilometers) and, third, an "intoxication picture" after

Marathon race when the percentages of polymorphonuclear cells and lympho cytes are normal   The first two types of change have been observed by others but not the third, as previous work has shown that the picture in the blood following Marathon races is that of a polymorphonuclear leukocytosis   That a lymphocytosis may follow a bout of short, intensive exercise is to be expected in some instances, because the exertion has been shown to increase the flow of lymph (Rous, 1908)   The original view of Ehrlich was that a lymphocytosis could result from an increase in the rate of production of the lymphocytes in lymphoid tissue and also from a flushing out of the lymph cells through an increase in the lymph flow   The classical observations of Rous confirm the view that the lymphocytes, too, like the polymorphonuclear cells, are sequestered but in the lymphatic rather than the circulatory system, and that following exercise they are redistributed to the circulating blood thereby causing an in crease in the percentage of these cells   The experiments of Rous prove the exist ence of a large reservoir of lymphocytes stored in the lymphatic system, which is quickly yielded to the blood following exercise

Thus in any person there are at least two ever present variables, which are responsible for the changes in the white blood cell content of the circulating blood   One of these is the reserve supply of polymorphonuclear leukocytes which is sequestered in the inactive capillaries of the body   The other is the segregation of lymphocytes in the lymphatic system. Cells of both types are returned to the circulating blood following muscular activity but in one person the lymphocytes may be swept into the circulation first, while in another it may be the polymorphonuclear cells   In general it may be said that the lympho cytes more commonly respond to short severe exercise whereas in prolonged exertion the leukocytosis is of the polymorphonuclear type

In the course of a comprehensive investigation of the problem of fatigue and hours of service of drivers of commercial vehicles operating in interstate com merce, Donahue of the Public Health Service (1941) observed the influence of driving upon the leukocyte picture of truck drivers

Preliminary studies indicated that the leukocyte count might be utilized as an index of the fatigue status of commercial truck operators   It was found that the average leukocyte count of 737 drivers was 9,811 per cubic millimeter, with a range varying from 4,900 to 19,200 per cubic millimeter   These figures were recognized as higher than those usually reported for the general population There was a statistically significant difference between the counts in different cities, for in Baltimore Chicago and Nashville the average counts were 9,010, 9 713 and 10,712 per cubic millimeter, respectively   This variation may have been due to a variation in the time of working hours, or different economic con ditions   No age trends were observed in the total leukocyte count   The mean values of the leukocyte counts of all drivers showed a consistent tendency toward an increase with hours of driving since major sleep   The rise was found to be relatively slight as compared with subjects undergoing severe muscular exercise   It was concluded that these results are suggestive of a chronic effect of the regime of the truck driver on the leukocyte level "

The effect of either the increased muscular activity associated with a pronounced tachycardia, or of the accelerated circulatory rate, or of both of these factors, is well demonstrated by the observations of Levine and Golden (1922) on patients with greatly increased heart rates   Eleven patients with paroxysmal rapid heart action were studied in whom the rate varied between 130 and 250 per minute for two hours to over twelve days   In six of these the leukocytosis ranged between 13,000 and 22,000 per cubic millimeter   The authors considered that the blood findings were due to the cardiac upset, as a return to normal occurred promptly when the rate was lowered

Bookman and Fraad (1935) studied the effect of prolonged training on the neutrophil count   They made weekly observations on 14 members of a college football team during the period of approximately eleven weeks of almost daily strenuous exercise   No significant variations were found in the percentage of neutrophils and hence it was concluded that prolonged exercise and training do not have an appreciable effect on the polymorphonuclear counts   They emphasize that this is rather remarkable as the total white blood cell count during energetic practice and actual contests must have fluctuated violently

VARIATIONS WITH AGE   According to Poncher (1943) the average leukocyte count shortly after birth is 25,000 cells per cubic millimeter   The level falls rather precipitately to about 14,000 at the tenth day of life, with a subsequent very gradual decline throughout infancy and childhood   Poncher's conclusions appear to be based, in part, on the observations of Kato (1935)   Washburn (1935) reported a total of 73 leukocyte counts on six babies performed during the first ten days of life   Wide variations were found both between different infants, and between counts on the same babies, the average of all the counts was 15,208 per cubic millimeter   For seven infants between two and twenty-six weeks of age a total of 535 leukocyte counts varied between 5,000 and 24,000, with 80 per cent of the counts within the range of 8,000 to 16,500   Washburn observed no tendency to orderly rhythms in the fluctuations of the individual infants' counts, nor was any correlation noted between the counts and activity or feeding   Kato (1935) analyzed 1,081 total and differential counts performed on 1,037 individuals between birth and fifteen years of age   At the end of the first day of life he found an average leukocyte count of 22,000 per cubic millimeter with a pronounced drop at the third or fourth day to an average of 8,750   Thereafter a slight temporary rise occurred during the first three months of life with an average maximum level of 10,000 to 11,000   A gradual fall was then observed, persisting throughout the remaining years of infancy and childhood

The values usually given for the leukocytes in normal infants and children are criticized by Osgood as being derived from studies on subjects not truly healthy   With a number of collaborators (1939, 1941) he has reported total, differential and absolute leukocyte counts for new-born infants and for age groups above 4 years   During the first day of life the leukocyte count averaged 15,000 with maximum values of about 24,000   A fall to an average of 10,000 was observed on the second day with a further decline to 7,000 on the third, fourth, fifth and sixth days   The count then increased to about 8,000 where

it persisted throughout the remainder of the ten day period of observation upon which the study was based   In a study of 86 children four to seven years old, Osgood and his associates found an average leukocyte count of 10,400 with a 95 per cent range of 5,500 to 15,500   Scatter diagrams indicated that, within these ages, the values for white blood cells are constant   At eight years the total leukocyte count declines, according to these authors, to an average level of 8,400, range 4,000 to 13,000, and these values persist through adolescence A further decrease occurs after the age of eighteen, when the adult values were established as mean total leukocyte count 7,400, range 4,000 to 11,000   The authors observed no differences between the sexes, either in total or differential leukocyte counts, in any of the age groups studied

The white blood cell values at four periods in the first year of life were studied by Magnusson (1938)   He provides an extensive bibliography of articles published prior to 1936   His own observations made on carefully selected subjects are shown in table 2

TABLE 2

AGE	NUMBER OF COUNTS	LEUKOCYTES (THOUSANDS PER CU MM.)	
		Mean	Standard deviation
weeks			
3rd	16	14 18 ± 0 77	3 08 ± 0 54
11th	20	10 79 ± 0 62	2 77 ± 0 44
23rd-25th	20	13 41 ± 0 62	2 77 ± 0 44
49th-52nd	20	12 82 ± 0 72	3 23 ± 0 51

In a study of approximately 300 healthy infants, aged 4 to 6 months, Bethell (1943) found an average leukocyte count of 11,279, standard deviation of 1,012 cells per cubic millimeter   At 10 to 14 months the mean count was 12,358, standard deviation 3,886   In the older age group the flatness of the frequency distribution curve, with some skewness toward the higher values, may be due to the inadvertent inclusion of children with subclinical pathologic processes If so, it illustrates the difficulty involved in the selection of normal subjects for the determination of leukocyte counts applying to young children   Relatively mild infectious processes may produce either leukocytosis or leukopenia, depending upon the responsible etiologic agent, and such changes tend to persist for long periods after the disappearance of clinical evidences of disease

At birth the neutrophils are the predominant type of leukocyte in the circulating blood   Their relative and absolute numbers fall rapidly, and during the second week are exceeded by the lymphocytes. After the first year the lymphocytes gradually decline but remain the most numerous cells until about the fourth year when they are surpassed by the increasing percentage of neutrophils According to Poncher (1943) the number of granulocytic and lymphocytic types approximate each other at the tenth day of life and again at the fourth year Eosinophils and basophils remain at a relatively uniform level throughout infancy and childhood and their numbers are about equal to the adult values

TABLE 3

Mean leukocyte values at different ages*

AGE	AUTHOR	TOTAL W.B C 000/CU MM	NEUTRO-PHILS	EOSINO-PHILS	BASO-PHILS	LYMPHO-CYTES	MONO-CYTES
			per cent	*per cent*	*per cent*		
Newborn	Poncher† 1943	25,000	60 0	2 5	0 5	22 5	10 0
	Chuinard‡ 1941	15,000	60 0	3 0	0 5	30 0	5 0
10th day	Poncher†	14,000	40 0	2 5	0 5	42 5	7 0
	Chuinard‡	8,000	43 0	3 0	0 5	45 0	5 0
4th–6th month	Poncher†	12,000	32 0	2 5	0 5	52 5	4 0
	Magnusson 1938	13,410	24 18	3 09	0 21	64 16	7 32
	Bethell 1943	11,279	25 0	1 80	0 15	69 33	3 61
10–12th month	Poncher†	9,000	32 0	2 5	0 5	57 0	4 0
	Magnusson	12,820	29 52	2 54	0 25	60 63	6 41
	Bethell	12,358	28 62	2 46	0 14	65 54	3 65
	Suzuki§ 1937	11,000	27 5	2 0	0 6	62 5	5 0
4th year	Poncher†	9,000	42 0	2 5	0 5	45 0	4 0
	Osgood‡ 1939	10,400	41 0	3 0	0 5	48 0	3 0
	Suzuki§	8,500	45 0	2 5	0 6	45 0	5 5
7th year	Poncher†	8,000	50 0	2 5	0 5	33 0	4 0
	Osgood‡	10,400	41 0	2 0	0 5	48 0	3 0
	Suzuki§	8,000	50 0	2 5	0 6	40 0	5 5
12th year	Poncher†	8,000	52 0	2 5	0 5	28 0	4 0
	Osgood‡	8,400	41 0	2 0	0 5	48 0	3 0
	Suzuki§	7,000	60 0	3 0	0 7	30 0	6 0
15th year	Osgood‡	8,400	51 0	2 0	0 5	42 0	4 0
	Suzuki§	6,500	65 0	3 0	0 7	23 0	6 0
Adult	Osgood‡	7,400	55 0	2 0	0 5	38 0	4 0
	Suzuki§	6,000	67 0	3 0	0 7	23 0	6 0

* It has not been possible to establish statistically comparable ranges for the data included in this table   It is designed to indicate the trend of change throughout infancy and childhood   Apparently healthy individuals, especially in infancy, may give values differing widely from the means   Where the summation of percentages falls appreciably short of 100, the discrepancy is due to omission of unidentified and disintegrated cells   The numbers of observations from which these figures were derived are comparatively large with the exception of those of Magnusson   This author, however chose his subjects with especial care and subjected his data to detailed statistical analysis

† Approximate values calculated from the authors' ranges

‡ Smoothed means, with some values adapted from the authors' classification to fit the present table

§ Data largely compiled from the reports of many authors, chiefly in the Japanese and German literature

The mean values for the total and differential count throughout infancy, childhood, adolescence and adulthood, as observed by several recent authors, are given in table 3

PREGNANCY, LABOR AND THE PUERPERIUM   Most observers report an elevated leukocyte count during pregnancy, the increase being due to greater numbers of neutrophils, with a higher percentage of younger forms than is found in non pregnant women   Carey and Litzenberg (1936) report 977 leukocyte counts obtained during the course of 134 pregnancies   The median value of these counts was between 10,000 and 11,000 per cubic millimeter, with no consistent alterations throughout the duration of gestation   Fifty per cent of the counts fell in the range of 8,700 to 12,500   No differences in leukocyte counts between primiparae and multiparae were observed   Hematologic data

TABLE 4

Leukocyte values in pregnancy the puerperium and at approximately one year after delivery
(Bethell Hartsuff and Farrell 1943)

MONTH OF PREGNANCY	LEUKOCYTES (THOUSANDS/CU.MM.)			DIFFERENTIAL COUNTS (MEAN PERCENTAGES)					
	No of subjects	Mean	S.D	No of subjects	Neut.	Eos.	Bas.	Lymph	Mono
2nd	11	10 182	2 886	10	58 00	2 00	0 00	34 00	4 90
3rd	46	10 023	2 445	41	66 71	1 67	0 17	27 85	3 93
4th	81	10 420	2 154	79	67 66	2 17	0 11	27 51	3 35
5th	125	10 888	3 112	114	67 72	1 68	0 15	26 06	4 11
6th	154	10 539	2 543	147	69 56	1 40	0 12	25 21	3 98
7th	200	10 875	2 435	188	69 68	1 39	0 15	25 30	4 41
8th	226	10 518	2 975	190	69 47	1 58	0 12	25 11	4 47
9th	174	10 339	2 515	149	68 96	1 52	0 09	25 32	4 46
Postpartum									
5th–9th week	343	8 192	1 858	334	51 29	2 84	0 31	41 28	4 26
10th–14th month	111	8 072	610	107	54 63	2 54	0 26	39 52	3 83

obtained on 36 women at different periods of gestation were reported by Olivella, Chediak and Ballestero (1938)   They found average values for the total leukocyte count of 9,250 cells per cubic millimeter neutrophils, 68 per cent, eosinophils, 2 per cent, lymphocytes, 23 per cent, monocytes, 4 5 per cent.

Blood studies were carried out by Bethell, Hartsuff and Farrell (1943) on healthy women in various months of their pregnancies, at approximately six weeks postpartum, and at about one year after delivery   In all, 1471 total leukocyte counts and 1359 differential counts were obtained   Although statistical analysis of these data has not been completed it is possible to observe the general nature of the leukocyte changes in pregnancy and the puerperium as evidenced by the figures in table 4   The total leukocyte count remains constantly elevated throughout pregnancy with relatively minor changes from month to month   There is a persistent neutrophilia which increases through the seventh month of gestation and then appears to undergo a slight decrease.

Lymphocyte, eosinophil and basophil percentages are lower than in non-pregnant healthy adults, but the absolute values for these cells are but little affected during gestation   The percentage of monocytes is approximately the same in pregnant and in non-pregnant persons, but their absolute number is increased in the former group   Throughout pregnancy the range of leukocyte values is very great as indicated by the high figures for the standard deviations   The total count declines and becomes more uniform at the 5th to 9th week postpartum, the decrease being at the expense of the neutrophils   The data obtained at approximately one year after delivery may be considered as representing the normal non-pregnant leukocyte values for the subjects of this investigation

Changes in the leukocyte count during labor have been studied by Gibson (1937) who observed a slight rise in total cell values at the beginning of parturition and a much greater increase immediately after its termination   Counts in the range of 10,000 to 39,800 were found during the first six hours after delivery in 38 normal women   In primiparae the leukocyte values tended to be higher and the leukocytosis to persist for a longer time than in multiparae   The leukocytosis of parturition was made the subject of an investigation by Boyd, Blenkinsop and Mylks (1937), with particular reference to the lipid composition of the white blood cells   In 14 patients the average leukocyte count in early labor was 7,100 cells per cubic millimeter, the lowest being 4,300 and the highest 10,000   At the end of labor, but before anesthesia, the average was 8,000, with a range of 5,700 to 11,200   In every instance some increase in the count occurred, averaging 1,700 cells, a 24 per cent gain over the values early in labor   No changes either in the differential count or in the lipid composition of the cells between beginning and advanced labor were observed   Earlier publications on the leukocyte count in labor have been reviewed by Wolff (1941), who reports studies on fifty normal women observed from shortly after the onset of labor until the conclusion of hospitalization at the eighth day after delivery   The number was equally divided between primiparae and multiparae   Twenty-one of the subjects were also examined at some time during the last weeks of pregnancy when their leukocyte values averaged 8,054 cells per cubic millimeter, with a range of 5,750 to 12,200   In all of these women a rise averaging about 2,000 cells was noted after the onset of labor, as compared to the previous counts   As labor progressed the leukocyte values increased to maximum averages at delivery of 22,250 and 15,270 in the cases of primiparae and multiparae respectively   The third stage of normal labor was not associated with further changes in the counts   The leukocyte values gradually returned to a normal range by the seventh day of the puerperium   Wolff states "that the work of the contracting uterine muscle appears to stimulate the mobilizing of the polymorphonuclear leukocytes into the systemic circulation "

EMOTION   Leukocyte alterations, both quantitative and qualitative, associated with affected states have been reported by many investigators   The subject is discussed extensively by Garrey and Bryan (1935)   In rabbits, including splenectomized animals, Nice and Katz (1936) observed, consistently,

leukopenia occurring during excitement, whereas in cats the same stimulus was followed by leukocytosis   In another article Katz and Nice (1936) reported no change in the ratio of non filamentous to filamentous neutrophils in the blood of rabbits during excitement.   They conclude that the emotional leukopenia of rabbits appears to be due to the sequestration of neutrophils in the peripheral capillaries and tissues

In humans with emotional disorders Milhorat, Small and Diethelm (1942) observed the frequent occurrence of leukocytosis   The subjects of their investigation comprised 200 psychiatric patients and no correlation was noted between the level of the white cell count and the specific psychiatric disease, although the degree of leukocytosis paralleled to a certain extent the intensity of the emotional reaction   Fear, agitation or anger characterized the emotional states, associated with leukocytosis   Improvement in the subjective reaction was followed by a return of the leukocyte count to normal levels   Although no consistent changes, either in the total or differential leukocyte values, were observed by Beccle (1935) in a study of 20 psychotic patients, he observed an increase in the percentage of cells containing ' toxic" (basophilic with Roman owsky stain) and Sudanophile granulations.   The extent of such granulations varied directly with the intensity of the mental disorder   Hematologic values, including total leukocyte and differential counts, were determined by Hill and Taylor (1938) in 21 subjects with pathologic anxiety states   The results were subjected to statistical analysis and showed no significant differences from the data afforded by a control group   However, in comment, it appears from the authors' protocol that their subjects were not in a state of severe emotional stress at the time of examination

THE EFFECT OF DIETARY CHANGES AND STARVATION   A physiologic study by Forbes, Johnson and Consolazio (1941) of the blood of share croppers in Mississippi showed that a majority of the apparently healthy negroes who were observed had a leukopenia with related neutropenia, whereas white persons living under the same conditions had normal white blood cell counts   The average leukocyte count of 23 negroes was 4,050 cells per cubic millimeter, whereas the average count of 7 white persons living under the same conditions, and said to be partaking of similar diets, was 7,600 per cubic millimeter   Some of the percentages of neutrophils in the negroes were as low as 22 to 34, and the average for the entire group was 50 per cent, while the average percentage in the white persons was 60   Iron medication caused an increase of the neutrophils in the blood of 18 negroes from an average of 1500 to one of 2700 per cubic millimeter'   The authors state that the cause of the leukopenia is not apparent That a dietary deficiency might have been an important etiological factor is a possibility but the evidence in regard to this is not clear   It was difficult to obtain an accurate picture of the eating habits of the negroes but there did not appear to be a pronounced dietary deficiency   Nevertheless, it is admitted that "occasionally they might be reduced to corn, beans, potatoes and salt pork "   That the leukopenia may possibly be associated with a food deficiency is supported by the experimental work of Day and his associates (1940)   It was

observed by these investigators that a leukopenia as low as 700 cells per cubic millimeter occurred in monkeys when they were fed the Goldberger black-tongue-producing diet

Benedict and his collaborators, as reported in an extensive study on human vitality and efficiency under prolonged restricted diet (1919), made certain observations on the blood in normal subjects who received such a diet for intervals varying from 22 to 37 days   The food intake was such that approximately a 10 per cent weight loss was attained   Under this regimen there was no significant change in the total white blood cell counts   It was considered that there was a disturbance of the proportion of the various types of white blood cells, as the average per cent of lymphocytes was high (36 per cent), and the average of the neutrophils was low (56 per cent)   In New England, at that time, the average normal percentages for lymphocytes was considered to be 22, and of neutrophils 64

Early observations on the effect of fasting in man and animals on the morphological constituents of the blood have been extensively reviewed by Ash and reported by Benedict in his remarkable study of the changes occurring during prolonged fasting (1915)   Unfortunately many of these reports are obviously marred by faulty technic and must be rejected   Almost every type of numerical change in the leukocytes has been reported   The results of Ash, however, can be accepted as accurate   He made a complete blood study on a healthy male who lived for thirty-one days without food and drinking only distilled water It was concluded that in an otherwise healthy person, with restriction of mental and physical activities, the blood can withstand complete abstinence from food for a period of at least 31 days, without displaying any essentially pathological changes   There were some significant alterations, however, in the leukocytes which were interpreted as physiological   Their number increased rather rapidly at the onset of the fast, reaching 12,400 per cubic millimeter on the third day   There was a fall to 8,400 per cubic millimeter on the fourth day, after which there was a variation of about 1,000 per cubic millimeter until the 16th day when the count approximated the preliminary level   Observations could be made only for three days after the termination of the fast, and no important changes were noted during this interval   The fluctuations in the total leukocyte count were obviously due to changes in the number of the neutrophils   Ash had no explanation for the increase of neutrophils during the early days of the fast other than, as he says, a rather unscientific one, namely, that the neutrophils are ever on the defense for the organism, and that these cells are most sensitive to changes in body conditions   The increase cannot be attributed to alterations in the water content of the blood, to an obscure bacterial infection, or to a stimulating effect of the perverted products of metabolism, for there was no evidence that these factors were of importance   The paucity of accurate observations which deal with the relationship between the food intake and changes in the leukocytes, makes any generalization concerning this subject unwarranted   From the studies carried out under the direction of Benedict it does not appear likely that starvation and undernutrition result

in any significant changes   The observation of a low white blood cell count in negroes, and the leukopenia seen in animals which are given a black tongue-producing diet, suggest the possible relationship between some components of the diet and the level of the total white blood cell count.   Obviously the subject demands further careful studies before conclusions can be formulated

DIGESTION   Whether or not there occurs a leukocytosis attributable to digestion is still a controversial question   The very extensive literature on the subject is reviewed by Arneth and Ostersdorf (1923) and Garrey and Bryan (1935)   Within recent years comparatively few contributions to the subject have been made, and it is the consensus of most observers that diurnal variations of the leukocyte count are not dependent upon digestive processes   Inada (1936) observed leukocytosis in rabbits after the intravenous injection of a five per cent solution of glucose (0 1 gram per kilo body weight)   The change in the leukocyte count was attributed to redistribution of the cells and was of the same type observed after feeding a diet high in carbohydrate or protein   Injection of minute fat globules caused no change in the white blood cells   Inada infers from his results that digestion leukocytosis in rabbits is related to the absorption of glucose, but is independent of the absorption of fat

THE NORMAL LEUKOCYTE PICTURE IN A HOT CLIMATE   Heat and intense solar radiation are factors common to the subtropical environment of Iraq in which the leukocyte picture in healthy British soldiers was studied by Kennedy and MacKay (1936)   They made the following observations 1, there was a relative reduction in the percentage of neutrophils, the mean being 56 6 with a minimum of 35 and a maximum of 75 5 per cent, 2, the monocytes were increased, the mean being 13 7, the minimum 4 0, and the maximum 29 5 per cent, 3, and the polymorphonuclear index showed a shift in the direction of immaturity   In addition, occasional abnormal cells were observed such as myeloblasts, promyelocytes, myelocytes lymphoblasts, Türk cells and normoblasts   The average blood counts were within the normal range as follows   Red blood cells, 5,375 000 per cubic millimeter, white blood cells, 8,780 per cubic milli meter, hemoglobin 96.3 per cent (Sahli)   The authors conclude that the only environmental agency affecting all subjects was the climate and that heat and solar radiation are the common factors to which the blood changes might be attributed   Heat may produce a transient increase in the circulating leukocytes, but its long continued action is unknown   It has been recognized that ultra violet radiation produces changes in the circulating blood (Russell and Russell, 1927, Kennedy and Flint, 1930, Kennedy and Thompson, 1927)   Russell and Russell (1928) have reported that sunlight, ultraviolet radiation and heat all produce an increase in the lymphocytes, and also that eosinophils are increased by irradiation   These authors state that when soldiers are transferred from a temperate climate to the tropics there is a considerable increase in the lymphocytes and erythrocytes, and the neutrophils are diminished

EFFECT OF HYPERPYREXIA UPON LEUKOCYTE COUNT   Changes in the leukocyte count were observed by Bierman (1934) following hyperpyrexia induced by exposure of patient to radiations with a wave length of about 30 meters

In most instances the rectal temperature reached 104 to 106°F and was maintained at this level for 3 to 4 hours by surrounding the patient with a hood containing carbon filament lamps   Hourly observations of the leukocyte count were made on patients suffering from various diseases   The initial change was a reduction of between 25 and 30 per cent in the total number of leukocytes which occurred in the first or second hours of treatment   This was due to a diminution in the neutrophils   Following this there occurred constantly an elevation in leukocytes whose maximum, amounting to approximately 80 per cent of the initial figures, occurred about the sixth to the ninth hour   The magnitude of the leukocyte response depended upon the height and duration of the temperature rise, but the maximum leukocytosis occurred several hours after the temperature had returned to the normal level   The highest leukocyte count recorded was 22,600 per cubic millimeter   As the increase was due mainly to changes in the total number of neutrophils, and as the staff (non-filamented) neutrophils showed the greatest rise, usually being between 200 and 300 per cent, it was concluded that the increase was due to stimulation of the bone marrow   The reviewers would like to comment that although the evidence that stimulation of the bone marrow seems to be clear, nevertheless the accelerated circulation rate which accompanies hyperpyrexia must contribute something to the transient increase in the leukocyte count by causing a redistribution of white blood cells   It is stated (Bierman and Fishberg, 1934) that during hyperpyrexia the velocity of the blood flow may be increased more than 400 per cent

THE EFFECT OF METEOROLOGICAL ALTERATIONS   Variations in the leukocytes in relation to meteorological alterations have been studied by Berg (1938)   In a previous communication Petersen and he (1933) had observed that the average counts in winter were higher than those in summer, while the degree of fluctuation was greatest in the latter season   A continuation of the study had led the author to conclude that in normal persons, a period of heightened systolic and diastolic pressure is followed by an increase in neutrophils and eosinophils with the appearance of more immature forms, and an elevation of the total white cell count   It was also found that the level and the extent of the seasonal fluctuations are influenced by constitutional habitus   For example, a person with a pyknic habitus was reported as having the highest level and also showed the greatest degree of fluctuations, whereas the strongly sympathicotonic individual not only had the lowest level, but also the lowest degree of fluctuations   References are given to authors who have noted variations in levels and fluctuations in the counts with constitutional differences   Data are presented showing the correlation which is claimed to exist between variations in the level of the leukocyte count, the character of the fluctuations, meteorologic episodes, and the pathologic condition of five patients suffering, respectively, with aleukemic lymphadenosis, myelogeneous leukemia, pulmonary tuberculosis (two patients), and chronic lymphatic leukemia

THE EFFECT OF ALTITUDE   In 1933 Stammers studied the white blood cell counts of persons living in Johannesburg, South Africa, which has an altitude of approximately 6,000 feet and receives 73 per cent of sun light   He reported

that the mean per cent of the polymorphonuclear cells was 54.2 and of the lymphocytes 39 7 These values are about 14 per cent lower than the average normal percentage for the neutrophils and approximately 14 per cent higher in the case of the lymphocytes, according to the standards then accepted As persons residing at high altitudes are exposed to more ultraviolet radiation, which had been shown by Clark (1922) to stimulate a relative lymphocytosis, it was assumed that this was the explanation of the changes observed

Peterson and Peterson (1934) reported their study on healthy young adults in the vicinity of Butte, Montana, at an elevation of 5755 feet, with a sunlight percentage of 57 Their conclusions agreed with those of previous observers, namely, that a relative lymphocytosis occurs at high altitudes, as the mean lymphocyte percentage was 36 2, and that of the neutrophils 54.36 These findings are in accord with those of Ruppanner (1920) who studied the blood of persons in the Swiss Alps, and with those of Hartman whose observations were made on members of the German Expedition to the Himalayas (1931)

Changes in leukocytes attributable to high altitude and increased solar radiation show, at the most, only slight deviations from generally accepted normal values, although the trend of such changes appears to be suggestive

DISPLACEMENT, DISTRIBUTION OR PSEUDO-LEUKOCYTOSES Vejlens (1938) in his comprehensive and scholarly review on the distribution of leukocytes in the vascular system contributes pertinent information relative to increases in the leukocytes in both physiologic and pathologic conditions His observations indicate that normally in the veins some of the white corpuscles are axial flowing and others are marginal and adherent to the vessel wall These latter cells are always neutrophils He believes that an increase or decrease in the number of marginal leukocytes in the capillary veins is the main reason for changes in distribution of the leukocytes The position of the leukocytes in the blood stream of the small veins is dependent upon two chief factors, first, alterations in the circulatory rate, and second variations in the suspension stability of the blood plasma which is probably related in part, at least, to its fibrinogen content. According to this theory a decrease in the white blood cell count results when there is a reduction in the rate of blood flow and consequently the corpuscles remain in the marginal position and many become attached loosely to the vessel wall With an increased circulatory velocity the number of white blood cells adherent to the wall of the small veins is diminished, and as they enter again the general circulation, the leukocyte count in the peripheral blood is increased The exact relationship of the changes in the suspension stability to the physiologic leukocytoses is less clear Doubtless in certain pathologic conditions such as infections, there is an increase in fibrinogen which causes a general marginal position in the veins This, Vejlens considers, is due to an increase in the adhesiveness between the neutrophils and the vein wall, and in a reversal of the size of the red and white corpuscles The latter is explained by the possibility that an increase in the fibrinogen content of the circulating blood causes an aggregation of red blood cells which are larger than single neutrophils or their aggregates

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SELENIUM POISONING[1]

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The element selenium was discovered by Berzelius and Gahn in 1817 while they were examining the sediment from a sulfuric acid plant at Gripsholm, Sweden Although the total production of selenium has never been very large, it has several important industrial uses, especially in the chemical, electrical, ceramic and metallurgical industries The yearly world production of selenium in peace time is about 1,000,000 pounds most of which is recovered from the anode slimes of copper refineries Watkins, Bearse and Shutt (180) have recently presented an excellent review on the commercial importance of selenium.

Selenium compounds are of importance in carrying out certain chemical reactions Selenium has been useful as a dehydrogenating agent in determining the structure of complex organic compounds (137) It has also been used as a specific oxidant (145) and as a reagent for ascorbic acid (89)

The greatest interest in selenium and its compounds has been aroused in relation to its toxic effects in the animal body Although Japha (70)[2] proved as early as 1842 that selenium was definitely toxic, it was not associated with general livestock poisoning ("alkali disease") until 1931

Probably the first authentic written record of selenium poisoning ("alkali disease") in livestock is the report written in 1856 by Madison (92), an Army surgeon stationed at Fort Randall, territory of Nebraska[3] He described a fatal disease among the horses which had grazed in a certain area near the fort The horses lost the long hair from the mane and tail, and their feet became so sore that they were unable to move about in search of food

Soon after the settlement of the territory around Fort Randall, about 1891, the farmers experienced difficulty with the same disease that Madison had described They called it the "alkali disease" because they associated it with alkali seeps and waters of high salt content. Even though experiments carried out at the South Dakota Experiment Station as early as 1912 and 1913 (83) (84) proved that suspected water did not cause the malady, the name "alkali disease" is still associated with the livestock disturbance which is now known to be chronic selenium poisoning

In 1929 Franke started his investigations at the South Dakota Experiment Station which led to co-operative work with several bureaus of the U S Depart-

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[1] Approved for publication by the Director of the South Dakota Agricultural Experiment Station

[2] Jones (Biochem J 4 405 1909) has credited Gmelin (54) with the first work on the effect of selenium on the animal body The reviewers have examined Gmelin's book and have failed to find any mention of selenium

[3] Part of the chapel at Fort Randall is still standing on its original site in southeastern Gregory county, South Dakota, just three or four miles from the Nebraska boundary

ment of Agriculture and the discovery, by Robinson (146), of selenium in cereals which Franke had found to be toxic to laboratory animals   A more detailed account of the early work on the selenium problem has been published (103)   Reviews on certain phases of the selenium problem have appeared at various times since the discovery of selenium in toxic grains (29) (60) (93) (97) (129) (154) (169) (173) (174) (180) (185)   The selenium problem as it is now recognized may be summarized briefly as follows   Soils which have been derived from certain geological formations contain selenium which is available to plants and is absorbed by the plants to the extent that they are toxic when consumed by animals   This toxic vegetation causes considerable livestock losses and is, directly and indirectly, a hazard to public health in certain areas

In this review the present status of the selenium poisoning problem will be discussed under the main divisions of   Geological Distribution of Selenium, Selenium in Soils, Absorption of Selenium by Plants, The Use of Selenium for the Control of Insects on Plants, Selenium Poisoning in Livestock, Selenium Poisoning in Poultry, Experimental Selenium Intoxication in Laboratory Animals, Organic Selenium Compounds, Public Health, and Methods of Analysis for Selenium

The Geological Distribution of Selenium   Franke et al (49) reported the observation that the cases of "alkali disease" of livestock encountered in their preliminary survey all occurred on "gumbo" soils derived from Pierre shales   Beath and co-workers (7) were the first to work on the geological distribution of selenium in relation to the poisoning of livestock   They had associated poisonous plants with Cretaceous shales before they found selenium in the plants Many of these plants grow either on the unweathered shale or soils derived from the shale

Cretaceous formations have contributed most of the selenium for the formation of soils which produce toxic vegetation (8) (9) (14) (15) (19) (103) (113) (114)   In South Dakota the Cretaceous formations have been mapped, sampled and analyzed for selenium (113) (114) and with the data thus obtained it is possible to predict, in some detail, areas which are capable of producing toxic vegetation   Stratigraphically the parent material which has produced most of the seleniferous soils in South Dakota (113) (114) (152) occurs near the top of the Pierre formation in the Mobridge member, at the base of the Pierre in the Sharron Springs member, and in the upper (Smoky Hill) member of the Niobrara formation which lies just below the Sharron Springs member of the Pierre formation

Highly seleniferous soils have been derived from the Mobridge member of the Pierre formation in northern Nebraska and southern South Dakota   Because of the stratigraphic position of this member of the Pierre formation it has been the parent material for soils over large areas   Fortunately, the selenium content of the soils is high only in the areas adjacent to the Missouri River in the south central part of South Dakota and in north central Nebraska (114)   To the west and north the selenium content of the Mobridge and the derived soils decreases to unimportant amounts   Soils which have been derived from the Mobridge member are of more value for crop production than the soils which have been

derived from any of the other members of the Pierre formation   Many of the soils derived from the other members are not suitable for cultivation and are used only for grazing purposes

The Sharron Springs member at the base of the Pierre formation contains considerable quantities of selenium in South Dakota, but its outcrops are limited to small areas and it does not weather readily   Therefore, very little productive soil has been derived from it in this area

The upper part of the Niobrara formation (Smoky Hill member) appears to be seleniferous and capable of producing toxic vegetation wherever it outcrops or occurs near the surface   Some of the most seleniferous soils in western South Dakota (113) (114) Wyoming (14) (15) (74), Kansas (15) and Colorado (14) (15) have been derived from the Smoky Hill member of the Niobrara formation

Selenium has been found in formations older than the Cretaceous formations, but in most cases it appears to be present in amounts insufficient to produce highly toxic soils or vegetation   Beath, Gilbert and Eppson (10) reported that formations of Permian and Triassic age contain selenium in Wyoming and Idaho

Williams, Lakin and Byers have reported that glacial drift contains selenium in North Dakota (190) and in Canada (190) (18)   During the past year a detailed study has been made of a glaciated area in South Dakota on which selenium ' indicator" plants have been found (164)   It was revealed that the selenium in this glacial drift occurs largely in glacial lake silts

Small amounts of selenium are continually being removed from seleniferous soils by leaching   The Colorado River carries appreciable quantities of selenium below irrigated areas where drainage and seep water come from seleniferous soils (186)   This selenium is carried to the Gulf of California   Selenium has been found in deposits from the bottom of the Gulf of Mexico (114) and in the sea-bottom deposits from various parts of the world (187) (15)   The fact that ocean water contains no more than a trace of selenium while marine deposits, both ancient (Cretaceous formations) and modern, contain selenium in appreciable quantities indicates that the element is precipitated upon reaching the ocean   The mechanism for the precipitation is suggested by the work of Strock (160), Byers et al (19) and Olson and Jensen (124) who found that selenite selenium is rapidly precipitated from solution in the presence of colloidal ferric hydroxide

Selenium has been found in small amounts in some meteorites from various parts of the world (17) and in rather large amounts in volcanic lavas from Hawaii (20)

Selenium in Soils   Moxon, Olson and Searight (114) have estimated that from 60 per cent to 80 per cent of the selenium in the original geological formation is lost in the soil forming processes   Thus, soils containing two to four parts per million of selenium have been formed from geological formations which originally contained about ten parts per million of selenium   The balance of the selenium is lost through leaching during the soil forming processes

Much of the work which has been done in locating seleniferous soils has been

of a reconnaissance nature   Geological formations which are known to be seleniferous and "indicator" plants (11) have been of great value in these surveys Byers and co-workers (14) (15) (19) (77) (189) (190) have made the most extensive surveys which have covered large areas in the United States and certain areas in Mexico (16) and Canada (18) (190)   In much of the reconnaissance work only samples of surface soil have been taken for analysis, but the intensive field studies of Olson et al (125) (126) (128) indicate that the selenium content of the surface soil is of little value in predicting the capability of the soil to produce toxic vegetation   The importance of sampling technique in survey work has been pointed out by Olson, Whitehead and Moxon (128)   Samples of soils and vegetation taken at two hundred foot intervals showed extremely wide variations in selenium content

Byers and co-workers (19) (188) have reported that selenium occurs in soils in the following forms   a, elemental, b, pyritic or selenide, c, selenite, d, selenate, and e, organic   Olson and Moxon (127) found that the humus of some surface soils contained up to 40 per cent of the total selenium   However, recent detailed field studies have shown that the selenium (organic and inorganic) content of the surface soil is of little importance as a source of selenium for plants   The water soluble selenates in the second and third feet of the profile are the important source of available selenium for grass plants   Some selenite was found in this water solution (128)

Lakin, Williams and Byers (79) have shown that there are large areas of highly seleniferous soils in Hawaii and Puerto Rico which do not produce toxic vegetation   They concluded that the selenium in these "non-toxic" soils occurs largely as a basic iron selenite which is not available to plants   Moxon, Olson and Searight (114) found that basic iron selenite was not readily available to plants in greenhouse experiments

Hurd-Karrer (65) (66) and Byers (14) from greenhouse experiments have reported that the sulfate content of soils has a great influence on the absorption of selenium by plants   In experiments carried out by the South Dakota Experiment Station (43) on field plots in a seleniferous area, the addition of sulfur failed to inhibit the absorption of selenium by crop plants   Hurd-Karrer (68) observed that sulfates would protect plants against selenates but not against selenites   Selenates constitute the main source of selenium available to plants at least in some highly seleniferous soils (128) but since the available selenium occurs in the second and third feet of the soil profile, it would be difficult to mix sulfates or other forms of sulfur into the soil thoroughly enough to be of much practical value   Furthermore, the seleniferous soils usually contain high concentrations of sulfates

Absorption of Selenium by Plants   Cameron (21) appears to be the first to have demonstrated that plants would absorb selenium from soils to which it had been added   He reported his work in 1880   In 1885 Knop (75) demonstrated that selenic and selenious acids are absorbed by plants   In 1934 Beath et al (6) reported that certain plants could be used as "indicators" for seleniferous soils and geological formations   These "indicator" plants are all classified

in the genera *Stanleya, Oonopsis, Xylorrhiza* and *Astragalus* (certain species)
Field observations (8) (9) (19) (103) suggested that selenium was essential for
the growth of the "indicator" plants, and Trelease and Trelease (176) have re-
ported that selenium is a stimulating if not an essential, element for "indicator"
plants   They have also studied physiological differences of "indicator" species,
and "non indicator" species of *Astragalus* (177)   Trelease (172) has classified
a number of species of Astragalus by germination tests in the presence and ab-
sence of sodium selenite   Beath, Gilbert and Eppson (11) have recently sum-
marized their extensive work with "indicator" plants

In range areas the highly seleniferous "indicator" plants are of importance
as a livestock hazard   In seleniferous farming areas, however, the "indicator"
plants are usually not as abundant as they are in grazing areas, and because of
the nature of the associated vegetation they are not especially important as a
livestock hazard   Crop plants and grasses are sources of selenium causing live-
stock losses in farming areas   While the selenium content of 'indicator' plants
often reaches several thousand parts per million, the selenium content of cereals
is seldom as much as one hundred parts per million

There has been no satisfactory explanation for the ability of "indicator" plants
to absorb such large amounts of selenium under conditions where ordinary plants
absorb only traces of the element   This certainly, should be a challenging prob-
lem for plant physiologists and plant biochemists

Robinson (147) determined the selenium content of wheat from various parts
of the world   A sample from South Africa contained 1 5 p.p.m.[4] which was
the highest amount found in imported samples, except for a sample from
Saskatchewan, Canada which contained 1 9 p p m.   Samples of wheat collected
from seleniferous areas in South Dakota contain up to 30 p.p.m. of selenium
and one sample of wheat which was grown on soils derived from the Niobrara
formation has been found to contain 63 p p.m of selenium   This is the highest
concentration of selenium in any sample of field grown wheat collected from
seleniferous areas   Hurd Karrer (67) produced wheat containing 220 0 p p.m.
of selenium by adding sodium selenate to soil in the greenhouse   The sample of
wheat which contained 63 p p.m of selenium was milled in an experimental
mill (115) and the fractions analyzed for selenium.   The bran contained 88 44
p p.m , the shorts 77 18 p p m and the patent flour 53.26 p p m. of selenium
The percentage distribution of selenium in the fractions of this milled sample
was comparable to the distribution of selenium in three other samples of wheat,
of lower selenium content, which were milled and analyzed at the same time.

Thorvaldson and Johnson (171) of the University of Saskatchewan analyzed
230 composite samples representing 2,230 individual samples of wheat, mainly
from the 1938 crop grown in the province of Saskatchewan   The maximum
selenium content of the composites was 1 5 p p.m   Individual samples were
found with a maximum of 4 0 p p.m. selenium

Byers and Lakin (18) analyzed a number of "indicator" plants and a few mis-
cellaneous plant samples from the provinces of Alberta, Saskatchewan and

[4] p p.m = parts per million

Manitoba, Canada   One sample of young wheat plants from Saskatchewan contained 120 p p m of selenium, another sample contained 30 p p m

Lakin and Byers (78) determined the selenium content of 951 samples of wheat and wheat products from Minnesota, North Dakota, South Dakota, Nebraska, Kansas, Colorado, Wyoming and Montana   Slightly more than 30 per cent of the samples collected in South Dakota contained over 4 0 p p m of selenium   Five per cent of the samples from Kansas contained over 4 0 p p m of selenium   It should be pointed out that their samples are by no means representative of wheat produced in states where seleniferous areas have been mapped, because they selected most of their samples in areas known to be highly seleniferous   If their samples had been picked at random like the samples collected in Canada (171) the average selenium content would have been much lower   This might well be illustrated by the values obtained by Moxon and Sebesta (119)   They analyzed 86 samples of wheat grown in 1939 which were taken at random from 14 counties in south central South Dakota   Seventy-two of these samples contained less than 1 0 p p m of selenium   Only one sample contained over 4 0 p p m , and it contained only 5,0 p p m   It would have been possible to select samples with higher concentrations of selenium Likewise, it should be possible to select individual samples high in selenium content in other states or in Canada after a study is made of the occurrence of selenium on many individual farms as has been done in South Dakota

Olson, Jornlin and Moxon (126) determined the selenium content of several grasses and plants collected over a three-year period from fenced plots on a highly seleniferous ranch   The results indicated that grasses containing from 10 p p m to 20 p p m of selenium will produce typical symptoms of chronic selenium poisoning ("alkali disease") in cattle

Perkins and King (136) found that selenium as sodium selenite when applied at rates up to 2 5 p p m of the soil in greenhouse experiments stimulated germination, spring growth and harvest weight of Tenmarq wheat   Applications of selenium greater than 8 0 p p m killed the wheat   Levine (87) and Stanford and Olson (165) have also observed that selenium in small amounts stimulated the growth of plants   For wheat plants grown in culture solutions (68), selenite selenium was more toxic than selenate selenium in the presence of high concentrations of sulfur while the toxicities were reversed if the sulfur concentration was below 30 p p.m

Painter and Franke (132) have studied the selenium sulfur relationships in cereal plants and concluded that although the metabolism of selenium and sulfur is similar in cereal plants, it is not identical for these two elements

The Use of Selenium for the Control of Insects on Plants   The toxicity of selenium to insects has led to the use of some selenium compounds as control measures   Sprays for the control of insects on citrus fruits have been prepared by dissolving selenium in a solution of potassium ammonium sulfide (55)   The United States Department of Agriculture in 1933 issued a warning regarding the use of selenium-containing spray materials, because little was known about the effects of the residues of such sprays (123)   In the same year the California

Agricultural Experiment Station began a study of the use of selenium-containing sprays on citrus fruits and grapes In 1938 the results of this study were reported by Hoskins, Boyce and Lamiman (64) The selenium-containing sprays were effective for the control of mites on citrus and grapes Based on the limited information available at that time, they concluded that if the selenium sprays were properly used there would be no danger to public health, in spite of the relatively high selenium content of the grapes

Hurd Karrer and Poos (69) found that aphids are killed by concentrations of selenium in wheat plants that are too low to cause injury to the plants Experiments carried out in Trinidad (138) have shown that cotton plants can be rendered toxic to the cotton stainer and the pink bollworm by the application of small amounts of sodium selenate to the soil Neiswander and Morris (122) have shown that the application of small amounts of selenium to soils will control red spiders on ornamental plants They observed significant reduction in both mites and other insects on plants which contained selenium.

On the basis of present information, the application of selenium to soils as a control measure for insects should be discouraged The concentrations of selenium applied are such that many plants could be made distinctly toxic

Even though some insects can be killed by adding selenium to the soil or by spraying the plants with selenium compounds, there are certain insects which thrive on highly seleniferous plants and seeds Trelease and Trelease (175) found weevils (*Acanthoscelides fraterculus*) and seed-chalids (*Bruchophagus mexicanus*) completing their life cycles in seeds of *Astragalus bisulcatus* The seeds contained 1,475 p p.m of selenium Byers et al (19) found flies (possibly *Pseudotephritis*) living on a sample of *Astragalus racemosus* plants which contained 1,800 p p.m of selenium Moxon (105) analyzed grasshoppers (*Melanoplus bivittatus*) which were collected from seleniferous plants They contained as much as 20 p p m of selenium and exhibited no deleterious effects from the seleniferous diet

Selenium Poisoning in Livestock Selenium poisoning of livestock has been divided into two general classes chronic and acute or sub acute (29) (103) The chronic type, "alkali disease " is predominant in South Dakota and in other states where seleniferous soils are farmed extensively It results from the consumption of vegetation, grains and forages containing up to approximately 25 0 p p.m of selenium for a period of several days or weeks The acute type, "blind staggers," is the common type in Wyoming and in the range areas of other states where highly seleniferous "indicator" plants are abundant These plants usually contain several thousand parts per million of selenium and frequently cause death within a short time when consumed in relatively small amounts by livestock.

Draize and Beath (29) and Beath et al (7) (8) have described and discussed the acute type of poisoning, 'blind staggers," in considerable detail The term "blind staggers" is a rather misleading designation because the animals may not become blind, and they may not stagger about in all cases In the early stages the animal may stray from the herd, there is usually a slight impairment of vision and the animal has difficulty in judging nearness of objects in its path.

In the next stage the blindness usually becomes more pronounced and is accompanied in most cases by a depraved appetite and the desire to chew wood, bone, metal objects, etc   There is also a greater tendency to wander, often aimlessly, in circles, and in case a solid object is encountered, the animal makes an effort to push forward rather than to turn to one side and go around the object   The last stage is characterized by various degrees of paralysis   There is evidence of abdominal pain, and death results from failure of respiration, much the same as has been observed in dogs and rats which have been injected with lethal doses of selenium (3) (39)   Some of the symptoms observed by Miller and Williams (98) in horses, mules and cattle which had been subjected to lethal doses of sodium selenite were similar to the symptoms of "blind staggers "

Symptoms of the "alkali disease" or chronic selenium poisoning have been observed in a number of "alkalied" animals (29) (103)   Dullness and lack of vitality is a general symptom   The animals become emaciated, stiff and lame, and fail to respond to good care and selenium-free feed   A prominent symptom in horses and mules is the loss of the long hair from the mane and tail   For this reason the chronic form of poisoning is often referred to as the "bobtailed disease "   This loss of hair from the mane and tail often takes place within a month after horses are moved to seleniferous areas   Madison (92) mentioned the fact that symptoms of the "alkali disease" appeared in dragoon (cavalry) horses brought to Fort Randall just ten days after they arrived from Fort Lookout and from Big Sioux River (eastern South Dakota)   Of special interest to the reviewers is the fact that the horses arrived at Fort Randall (from non-seleniferous areas) on August 10, since it has been our observation that symptoms of "alkali disease" usually start to appear at about this time of year in animals on seleniferous ranges   The appearance of the symptoms usually follows very closely the occurrence of hot winds which dry the green vegetation

The loss of long hair from the mane and tail in horses and mules, and the loss of the long hair from the switch of cattle is usually accompanied or followed shortly by lameness and soreness of the feet   Swelling appears at the coronary band   In very mild cases there may be no further change although the animals may be lame for some time   In severe cases a gradual separation of the wall of the hoof occurs below the coronary band, and a new growth of hoof starts at the coronary band   In some cases the old hoof is sloughed off and in others it remains attached to the new growth until the new hoof has grown to normal length   In cattle the old hoof usually remains attached to the new growth and in cases where there have been several attacks the old ragged hoofs may be 8 or 10 inches long and turned upwards at the end   During the time the animals are sloughing the old hoofs and growing new ones they are very lame and often are in severe pain   Usually they do not move about much and unless feed and water are within easy reach, death may result from starvation and thirst

As a result of placental transmission of selenium (183) animals may be born with hoofs which show the effects of selenium poisoning (103) (155)

Miller and Williams (99) produced chronic selenium poisoning in horses and mules by feeding small daily doses of sodium selenite over long periods of time

The symptoms were similar to those of the "alkali disease" but were not quite as pronounced

In hogs the symptoms of "alkali disease" are emaciation, lameness, loss of hair from the body and irregular growth of the hoofs with occasional sloughing of the hoofs similar to the condition in cattle and horses   When young pigs are fed corn containing 10 p.p.m to 15 p.p.m. of selenium, the symptoms appear within two or three weeks

Beath (5) has observed that sheep on seleniferous ranges produce lambs with abnormal eyes and deformed feet   In 1935 Draize and Beath (29) published data on 100 autopsies of cattle and sheep suffering from either "blind staggers" or "alkali disease "   This report covers both gross pathology and microscopic pathology   Following is a brief summary of their report.

Pathology of "blind staggers "   Atony of the smooth muscles of the gastro-intestinal tract, gall bladder and bladder is common   The blood vessels of the viscera are congested   In winter cases impaction of the rumen is usually observed   A stasis of the food material is evident also in the omasum.   The abomasum and upper small intestine show irritation of varying degrees and petechial hemorrhages and ulceration are noted in severe cases   The large intestine is usually free from irritation   Peritoneal blood vessels are congested and ascites is common

The liver is acutely congested and areas of focal necrosis appear early in the intoxication   These necrotic areas appear to be connective tissue scars which on contraction form pits upon the surface of the organ   Cirrhosis is not as prevalent in "blind staggers ' as it is in "alkali disease"   The gall bladder may be enlarged to twice normal size and the walls often exhibit mucoid degeneration and congestion of the blood vessels   There is congestion in the medulla of the kidney and kidney stones are not uncommon in the more advanced cases

Examination of the heart revealed endocarditis, myocarditis and petechial hemorrhages of the epicardium   Erosion of the long bones, especially the tibia, is found in about two-thirds of the cases   In typical cases of "blind staggers" the hoofs are not involved

"Alkali disease "   The lesions of "alklai disease" are of much more chronic nature than those of "blind staggers "   At autopsy the heart and liver show the most severe lesions   In the advanced cases the heart is invariably atrophied (dish rag) and shows evidence of decompensation   Bundles of muscle fibers appear to be separated by a sero-fibrinous exudate   Petechial hemorrhages on the epicardium are not as common as in "blind staggers" but endocarditis and myocarditis are prevalent.

The liver is cirrhotic and often atrophied   Microscopic examination reveals changes typical of cirrhosis.   The kidney is usually severely damaged in the "alkali disease" and glomerulo-nephritis is observed in many cases   Both the medulla and cortex of the adrenals are hemorrhagic   Lesions in the gastro-intestinal tract are of the same general character as observed in "blind staggers" except that the intense irritation is not observed   Erosion of the joints of the long bones is common in "alkali disease '

A high incidence of anemia is found in both ' alkali disease" and "blind stag

gers " The reviewers have observed that the hemoglobin content of the blood can be used to detect early stages of the "alkali disease" in cattle   In severe cases the hemoglobin may drop as low as 7 0 grams per cent (164)

Selenium Poisoning in Poultry   Poor hatchability of eggs has always been a common complaint among farmers in "alkalied" or seleniferous areas and this defect can be used as an aid in locating such areas   Hatchability is influenced by concentrations of selenium in the feeds that are too low to cause manifest symptoms of poisoning in other farm animals   Chicks that do hatch from seleniferous eggs are weak and have a wiry appearing down   Experimental work has shown that the developing chick embryo is extremely sensitive to selenium poisoning

Before selenium was suspected as the cause of "alkali disease," Franke and Tully (50) obtained eggs from a farm in an "alkalied" area and incubated them at the South Dakota Agricultural Experiment Station   Contrary to the popular belief, nearly all the eggs were fertile   Upon examination only 6 out of 133 eggs showed no signs of a developing embryo   However, only 5 chicks actually hatched, and 2 of these died within 2 days   The rest of the fertile eggs contained deformed embryos in various stages of development   Some of the monsters were alive but were unable to make their way from the shell because of their deformities   In most of the monsters the upper beak was very short or missing, many of the embryos had deformed legs and wings and some lacked eyes

Tully and Franke (178) then found that a ration containing 65 per cent of grain from an "alkalied" area inhibited growth of chicks, initial egg production of pullets was delayed and total egg production was reduced   Later, after Robinson (146) found selenium in the toxic wheat which Franke had supplied, it was shown (103) that inorganic selenium produced the same effects in chickens as had previously been noted from toxic grains   This work has also shown that chickens appear to be more sensitive to poisoning by inorganic selenium than white rats   Decreased feed intake and production of monsters in the eggs was noted when White Leghorn hens were fed a ration containing 3 5 p p m of selenium as sodium selenite

In the ration of young chicks 8 p p m of selenium as sodium selenite caused depression in growth, but 4 p p m had no apparent effect

As further evidence that the deformities of the embryos were caused by selenium Franke et al (38) injected small amounts of sodium selenite into the air cells of fertile eggs before incubation   Selenium in amounts as low as 0 0005 mgm per egg (0 1 p p m) caused typical deformed embryos

When rations containing seleniferous grains (15 p p m selenium in ration) were fed to laying hens for seven days the hatchability of the eggs decreased to zero but returned to normal in seven days after the hens were changed back to selenium-free rations (140)   One per cent of elemental sulfur in seleniferous rations failed to affect the toxic action of selenium

Poley and Moxon (139) studied the tolerance levels of selenium in laying rations   Selenium at a level of 2 5 p p m had no appreciable effect upon hatchability, at a level of 5 0 p p m hatchability was reduced slightly, and at a level of 10 0p p.m hatchability was reduced to zero   It has been recommended

that starting rations for chicks contain less than 5 p.p.m. of selenium (141)
The selenium content of meat and eggs from hens which had been fed a seleniferous ration for six weeks was reported by Moxon and Poley (116)   When the
ration contained more than 2 5 p.p.m. of selenium, the meat and eggs contained
concentrations of selenium in excess of the suggested tolerance limit for foods
(15) (121)

Landauer (80) studied the effect of chronic selenium intoxication in laying
Creeper hens on their progeny   He found that the Creeper mutation exaggerated the interference with normal development caused by selenium.   Except
for this study by Landauer, all of the published work on selenium poisoning in
poultry has been done at the South Dakota Agricultural Experiment Station

Experimental selenium intoxication in laboratory animals   The experimental
investigation of the action of selenium on the animal body has been stimulated
by two interests   The earliest interest was in the action of toxic doses of
selenium to show the pharmacological relationship of this element to other well
known poisons   The second interest is in the effect of relatively small amounts
of selenium ingested over a long period of time   It is now recognized that
chronic selenium poisoning of animals has been known for nearly a century,
but the possibility of widespread chronic selenosis in humans has been recognized
only in the past decade

Acute toxicity of selenium   Czapek and Weil (27) credit Japha (70) with the
first investigation of the toxicity of selenium   Japha reported in 1842 on the
action of metallic selenium and various selenium salts on men and animals
His subjects were chiefly local animals and himself   Rabuteau (143) in 1869
published the results of his investigations on the properties and elimination of
selenium (and tellurium) from the animal body   He contended that character
istic blood changes were produced, and that death was due to mechanical effect
of certain crystals formed in the blood

As the result of work extending over several years, Czapek and Weil (27)
reported the pharmacological action of toxic doses of selenium on cold blooded
animals (the frog) and warm blooded animals (rabbits, cats and dogs)   They
used sodium selenite and selenious acid as sources of selenium, assuming that
the selenates were toxic to the animal only after reduction to selenite.   The
principal results and conclusions of Czapek and Weil's work may be summarized
as follows

The lethal dose for the frog is less than 0 1 mgm of selenium   The essential
action is a narcosis of the central nervous system followed by stoppage of the
heart in an engorged condition in diastole   In general the action of the sodium
selenite on the heart was contrary to that of digitalis, and the two compounds
counteract each other to a certain extent   They concluded that sodium selen
ite acts on the ganglia of the heart or some other regulatory mechanism rather
than directly on the heart muscle itself   Death is due to a combination of the
effects on the heart and on the central nervous system   There was no dif
ference between the action of sodium selenite and sodium arsenite but the latter
seemed to kill frogs more quickly in the same dose

In warm blooded animals the first symptoms appear about 5 minutes after

gers " The reviewers have observed that the hemoglobin content of the blood can be used to detect early stages of the "alkali disease" in cattle   In severe cases the hemoglobin may drop as low as 7 0 grams per cent (164)

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namely 4 0 mgm per kgm   All reports agree that selenium metal is non toxic because of its insolubility

In determining the relative toxicity of arsenic, selenium, tellurium and vanadium Franke and Moxon (39) administered the sodium salts of all the elements by intraperitoneal injection   The relative toxicity in decreasing order was tellurite, selenite, vanadate, arsenite, selenate, arsenate and tellurate   No deaths were noted from 160 0 mgm /kgm. of molybdenum as ammonium molybdate

Smith, Stohlman and Lillie (159) noted that repeated small doses of selenium were cumulative in effect, although the amount required to kill in this manner was greater than in a single dose   They found no evidence of a tolerance being acquired, as had been reported by Modica (101)   Marked individual variation in susceptibility to selenium toxicity was noted for rats, cats, and rabbits, and the same has been reported for dogs (3)

The characteristic blood changes which Rabuteau (143) ascribed to selenium poisoning have not been observed by subsequent investigators (26) (27) (195)   The only recent study of the changes in the blood picture due to acute selenium poisoning is that of Anderson and Moxon (3)   They summarized the characteristic blood changes in dogs as follows   Marked rise in hemoglobin and as much as 62 per cent rise in hematocrit   Decrease in inorganic phosphorus, non-protein nitrogen, calcium, ascorbic acid, and blood sugar   Reduction in glutathione content of the blood was indicated   No effort was made to determine the presence of the characteristic crystals in the blood described by Rabuteau, but not found by other workers

Recently Moxon and Rhian (117) have investigated the effects of massive doses of selenium as sodium selenite (20 mgm /kgm) administered subcutaneously to dogs   It has been observed that death results in 25 to 30 minutes after administration of the selenium.   This observation may be used for comparison with other investigators' reports that doses of 4 mgm./kgm or more of selenium result in death "in a few minutes," or ' in a relatively short time "

That the toxicity of sodium selenite injected intraperitoneally can be influenced by other compounds was reported by DuBois, Rhian and Moxon (31)   Reduced glutathione, in a ratio of 10 moles to one mole of selenium, when injected two hours before the selenium prevented death in 8 of 10 rats given 3 0 to 3 5 mgm of selenium per kilogram   The same amount of glutathione was ineffective when 4 0 mgm /kgm of selenium was given, showing that large excesses of GSH are necessary for the protective effect

Dudley (34) has reported that selenium oxychloride is extremely vesicant and toxic   When 0 01 cc was applied to the skin of a rabbit death occurred in less than 24 hours   The reviewers have noted that one drop of the compound applied to the skin of a dog produced death in a few hours

Sub-acute and chronic toxicity of selenium   The comprehensive investigation of the chronic toxicity of selenium was begun by Franke in 1928 as stated earlier in this review   Since then many investigators have shown that symptoms produced by feeding naturally toxic (seleniferous) rations and normal

(selenium-free) rations with added inorganic selenium are virtually identical (40) (44) (46) (57) (94) (121) (150)   The more important symptoms of sub-acute selenium poisoning in the white rat described by Franke (36) and Franke and Potter (45) may be summarized as follows a, marked restriction of food intake, b, decreased growth, c, marked, progressive anemia, and d, definite pathological changes especially in the liver   Rats fed a diet with a selenium content which produces a condition of sub-acute toxicity restrict their food consumption to as little as 25 per cent of that of rats fed a similar diet without selenium   Most of the rats assume a characteristic hunched posture   In a few cases the hind legs become paralyzed   The fur becomes rough and is stained a dark yellow around the genitals   In rats that die in a short time, gross pathology is not evident   The most marked symptom is dilatation of the veins of the visceral region   The lungs and liver usually have a congested appearance   The reproductive organs are undeveloped and may show degeneration   The intestines show evidence of hemorrhage, the animals are jaundiced and very emaciated

Rats fed a ration with a lower selenium content may be said to show symptoms of chronic poisoning   They may show slight increases in weight and appear to be in fair condition, but their reproduction may be impaired (48)   In many cases a definite breakdown of vital organs takes place   They develop an anemia of progressive severity and die with hemoglobin levels as low as 2 0 grams per 100 cc blood   The most outstanding pathology is in the liver which is atrophied, necrotic, cirrhotic and hemorrhagic in varying degrees   In cases of active liver regeneration it is usually found that the right lateral and/or the caudate lobes are hypertrophied   The left lateral and central lobes are most susceptible to atrophy   In many cases the heart and the spleen are enlarged   Ascites and pleural edema are common   In some instances death results from internal hemorrhage from the liver   Lillie and Smith (91) have studied the histogenesis of the liver changes associated with chronic selenosis

Recognizing that there are individual variations it may be said that in general symptoms of chronic poisoning in rats are common when the ration contains 5 to 15 p p m of selenium, and sub-acute symptoms are produced when the ration contains 15 to 25 p p m selenium or more

The tendency for range animals to avoid certain plants, or the common vegetation of well-defined areas, has long been recognized in relation to "alkali disease "   An analogous situation has been shown to exist in laboratory feeding trials   Rats given a choice of rations invariably avoided those containing either naturally occurring selenium or sodium selenite (47)   Miller and Schoening (100) noted that young pigs were loath to eat corn containing selenium   Admittedly, the lower food intake of the animals accounts for some of the symptoms of selenium toxicity, but Franke and Potter (45), Franke and Painter (42) and Munsell, DeVaney and Kennedy (121) have shown that the other symptoms and at least part of the growth effect are due to the selenium in the ration

Relatively little experimental work on chronic selenium poisoning has been done with laboratory animals other than the rat   Smith, Westfall and Stohl-

man (161) (162), Smith, Stohlman and Lillie (159), and Westfall, Stohlman and Smith (183) have reported investigations using cats and rabbits Moxon (107) studied selenium poisoning in pigs under laboratory conditions and Rhian and Moxon (144) have used 34 dogs in studies on the toxicity of orally ingested selenium Ellis et al (35) have studied selenium poisoning in fishes which were kept in water containing small amounts of selenium

Smith and his co-workers have been especially interested in securing information on the chronic toxicity of selenium that may be directly applicable to interpretation of their survey data on selenium poisoning in humans They noted in some of their work (161) that the susceptibility of the cat, rabbit and rat to selenium poisoning decreased in that order The cat, in common with other carnivora, showed more intestinal symptoms than the rabbit It is also interesting that these investigators have found no anemia in cats suffering from chronic selenium poisoning In later reports (156) (157) they concluded that the species differences in susceptibility could be accounted for on the basis of the selenium protein ratio of the diet It is still questionable whether this conclusion can be applied to species differences mentioned in other reports

In their work with dogs, Rhian and Moxon (144) found that these animals exhibited most of the symptoms shown by the rat and many shown by larger (farm) animals In general the dog is more susceptible to chronic selenium poisoning than the rat

The principal symptoms of chronic or sub-acute selenium poisoning described for the dog are as follows

As shown by voluntary restriction of food intake and sub normal growth, as little as 7 2 p p.m of selenium in the ration in the natural form (in corn) and 10 p p m as added sodium selenite were toxic As added sodium selenite, 20 p p m. selenium in the ration induced almost complete refusal of food and death in a very short time This amount of selenium in the natural form produced severe nervous disorders (resembling blind staggers") Pathologically the liver and spleen were most severely affected Small localized hemorrhages were noted in the intestines Severe ascites was quite common The dogs became markedly emaciated and the hair became coarse and rough In the later stages of intoxication apathy was common

Chronic or sub-acute selenium poisoning has little effect on the commonly determined blood constituents of the dog, except hemoglobin Anemia in dogs becomes progressively more severe as the selenium poisoning progresses, terminal values for hemoglobin being as low as 3 5 grams per cent Phosphatase activity of the blood is increased and non protein nitrogen is slightly reduced

Distribution of selenium in the body The occurrence of selenium in the animal body has been shown to be related to the amount and form of selenium administered and length of time of administration Dudley (32) has studied the distribution of selenium in acute cases of poisoning and Smith, Westfall and Stohlman (161) have summarized the limited information available up to 1937, on the distribution of selenium in the animal body

In cases of acute selenium toxicity, there is excretion of selenium as a volatile,

very odoriferous compound in the breath   It has been claimed that this compound is methyl selenide (61), but definite proof of this is lacking (151)   Miller and Williams (98) obtained some information on the amount of selenium retained by horses, mules, pigs, and a calf in cases of acute poisoning   In general they found selenium in all tissues examined except hide and hair   The kidney, liver, lung and spleen invariably contained the most selenium, and its occurrence in less active tissues of the body was apparently in direct ratio to the time of survival

Smith, Westfall and Stohlman (162) studied the distribution of selenium in doses up to two-thirds of the minimum lethal dose   It was noted that as soon as 5 minutes after the intravenous injection of sodium selenite the concentration in the kidney exceeded that in the blood   The selenium content of the kidney and liver increased, and that of the blood decreased for 6 hours after which there was a continued gradual excretion of the selenium from all parts of the body   It was noted that in all cases the concentration of selenium in the red blood cells was greater than that in the blood plasma

Through the use of radioactive selenium McConnell (95) has studied the distribution of a single sub-toxic dose of sodium selenate in the body of the rat up to 96 hours after administration   A wide but varied distribution of selenium was found in the tissues examined   The greatest concentration of selenium was found in the liver, muscle, gastro-intestinal tract and blood   Lesser amounts of selenium were found in lung, spleen, heart and tumor and none was found after 24 hours in skin, fur, teeth or long bones   Small amounts were deposited in tumor tissue   Selenium was excreted chiefly through the kidney, only a small amount being excreted in the feces   In a later report (96) it was noted that 3 to 10 per cent of a single dose of selenium was exhaled in the first 24 hours   The volatile compound was not identified

The distribution of selenium in the animal body after chronic poisoning has been studied thoroughly by Dudley (32), Smith, Westfall and Stohlman (161), Munsell, DeVaney and Kennedy (121), Moxon (103), Rhian and Moxon (144) and Anderson and Moxon (1)

After oral administration of selenium to produce chronic poisoning, all the tissues of the body contain some selenium   To some extent, the amount and kind of selenium in the ration and the length of the period of administration influence the amounts in various tissues   Munsell, DeVaney and Kennedy (121) found that when rats were fed a seleniferous ration over a long period of time, the selenium content increased to a maximum during the first 4 to 8 weeks of feeding and remained constant thereafter   If the poisoning did not become increasingly severe, the excretion balanced the intake   Excretion takes place through three channels, the breath, urine and feces   Smith, Westfall and Stohlman (161) have shown that in the cat 50 to 80 per cent of the total selenium is excreted in the urine and from a trace up to 18 per cent in the feces

In the body the highest concentration of selenium is found in the liver, kidneys, spleen, pancreas, and lung   Other less active tissues contain relatively much less selenium   It was noted (162) that more selenium is retained in the

body when the ration contains naturally occurring organic selenium than when it contains sodium selenite. Conversely, urinary excretion of sodium selenite is greater. Using a ration containing 18 p p m selenium, Anderson and Moxon (1) using smaller concentrations of selenium than used by Smith, Westfall and Stohlman, noted no difference in retention of naturally occurring selenium and sodium selenite. Retention of selenium was a maximum during the first week of feeding, and steadily decreased after that time. When the rats were placed on a selenium free ration, most of the selenium was excreted from the body within two weeks after the change. Individual variation in selenium content of the tissues is very marked (144)

Data obtained by the reviewers and others (164) indicate that when cattle graze on naturally seleniferous plants, the distribution of selenium in the body is qualitatively the same as that noted for experimental animals. For eight steers that had been on seleniferous range for two seasons the average analysis of body tissues (on the fresh weight) showed a concentration of 3 05 p p.m selenium in the hoof, 2.2 p p.m in the kidney and 1.2 p p.m. in the liver. All the other parts of the carcass were lower in selenium content, the muscle containing 0 88 p p.m. For 30 steers that had been on the same range for three seasons the average selenium content of the liver was 5 7 p p m, the kidney 4 1 p p.m, and the muscle 3 0 p p.m.

The characteristic pathology of the stomach noted in chronic selenium poisoning has aroused interest in its effect on the function of this organ. Smith and Stohlman (158) have reviewed the meager literature on this subject up to 1940. In their own experiments they found that chronic selenium poisoning in rats and cats had no essential effect on free or total gastric acidity

From a study of the effects of chronic selenium poisoning on liver function in rabbits and cats, Smith, Westfall and Stohlman (163) concluded that liver function measured by the rose bengal, bilirubin and hippuric acid tests could be correlated with the structural changes in the liver. The rose bengal test was most sensitive in measuring liver dysfunction due to selenosis

A number of investigators have commented on the effect of selenium on blood sugar and liver glycogen. Wright summarized the information available to 1941 (194). From his own work he concluded that doses of sodium selenite greater than 5 0 mgm /kgm cause increase in blood sugar and over 10 0 mgm cause decrease in liver glycogen in the rat. In the rabbit, 3 0 mgm /kgm cause an increase in blood sugar, followed in the fasted rabbit by a sharp fall preceding death. He also noted that fasted rats injected with sodium selenite have a lowered glucose tolerance and delayed deposition of liver glycogen from the injected glucose. The observations of Svirbely (170) and Lardy and Moxon (82) suggest that ascorbic acid may play an important rôle in the detoxification of selenium

Effect of selenium on the blood Franke and Potter (45) observed that one of the symptoms of chronic selenium poisoning in rats was a progressive anemia. Terminal values as low as 2 0 grams per cent hemoglobin were noted. This effect was first noted when the ration contained naturally toxic grain and has

been shown to be caused also by rations containing sodium selenite (46) (150) Smith, Stohlman and Lillie (159) have further studied the anemia of the rat and observed that the chief characteristics are "relatively low hemoglobin compared to the red count, marked reticulocytosis which at times may be as high as 80 per cent, anisocytosis, polychromatophilia and normoblasts in greater or lesser numbers "—"The anemia when well developed is essentially microcytic and hypochromic " These same workers observed that cats seem to be less inclined to show anemia in chronic selenium poisoning   Only two out of fourteen cats given daily doses of 0 2 to 0 25 mgm selenium per kilogram showed definite anemia

Progressive anemia is a constant symptom of selenium poisoning in dogs (144)   Hemoglobin values of 4 0 grams per cent were noted in dogs that lived a relatively long time on a ration containing selenium   In other instances, terminal values of 3 5 grams per cent have been observed in young puppies severely affected by chronic selenium poisoning (117)

Aside from the characteristic anemia, chronic selenium poisoning of the dog seems to have no characteristic effect on the blood picture (144)   Preliminary data indicate an elevation of blood phosphatase values, and a slight reduction in non-protein nitrogen   (This effect on phosphatase is contrary to that observed by Bauer (4))

Observations on the blood picture of cattle grazing on seleniferous range have failed to reveal any pronounced changes except for the hemoglobin levels (164)

The effect of dietary factors on selenium poisoning   Early in the investigation of the "alkali disease," Franke and co-workers (103) attempted to find a dietary factor (or factors) that would prevent or reduce the toxicity of seleniferous rations   Various feeding trials with rats showed that changing the calcium and phosphorus content (2 8 per cent to 11 2 per cent tri-calcium phosphate) and the Ca P ratio of the ration (1 2 to 1 6) had no beneficial effect   That vitamins A and D had no close relationship to selenium poisoning was shown by feeding a ration containing up to 4 per cent of cod liver oil   Yeast as a source of vitamin B complex (up to 0 8 gram per rat per day as a supplement) had no beneficial effect   The combined effect of supplements of orange juice, dry yeast and cod liver oil gave improved growth to rats but did not change the characteristic pathology due to selenium   In more recent work (164) it has been noted that daily administration of crystalline vitamin B_1 (either orally or by injection) to rats fed a ration containing 12 p p m of selenium greatly increased the severity of the symptoms

Cystine to the extent of 5 per cent of the ration had no alleviating effects on selenium poisoning and at the higher level seemed to be slightly toxic itself Schneider (150) also fed cystine at concentrations of 0 2, 0 4, and 0 6 per cent of the ration and noted no beneficial results   Sulfur fed at 0 5 per cent of the ration was not beneficial in selenium poisoning and itself was slightly detrimental to rats and chickens (103)

The effect of the protein content of the ration on selenium poisoning has been investigated (56) (90) (103) (153) (157)   From feeding rations of high, normal,

and low protein content (with equal calorific value) it was conclud
South Dakota workers (103) that selenium poisoning was less seve
ration with a high protein content. This conclusion has been substan
Smith (153) and Smith and Stohlman (157) who have extended the obs
as a possible explanation for certain previously observed species differ
susceptibility to selenium poisoning Expressed on the basis of per cent
in the ration and micrograms of selenium per 100 grams of ration, they con
that a ration with a protein selenium ratio of 1 30 or less is non toxic w
ration with the same selenium content and a protein selenium ratio of
is dangerously toxic Contrary to the finding of Smith and Stohlman (157
neither cystine nor methionine were themselves of value in alleviating the to
of selenium, Schultz and Lewis (151) found that addition of methionine
methionine deficient ration did have beneficial effects Probably the pr
effect noted is due to more than the methionine content of the diet (157)
proteins which, it is claimed, have definite value in the alleviation or prevent
of chronic selenium poisoning are casein, lactalbumin ovalbumin, gelatin, p
teins derived from wheat, dried brewers' yeast, desiccated liver, and zein T
workers mentioned above do not agree on the action of all these proteins
Of further interest is the observation reported by Moxon (108) that of con
mercial proteins fed to rats crude casein and linseed meal were the only ones tha
prevented the characteristic liver lesions Crude casein gave excellent growth
response and linseed meal gave the best growth of the vegetable proteins used,
but not as good as casein or dried liver Dried whole beef liver, Labco (purified)
casein, and whole milk powder all gave good growth, but failed to prevent or
alleviate pathological lesions of selenium poisoning Meat scraps, tankage and
corn gluten meal gave poor growth and no protection against selenium poisoning
In experiments with dogs it was noted that dried beef liver (18 per cent of the
ration) gave excellent growth and prevented all the symptoms of selenium poison
ing on a ration containing 10 p p.m selenium Crude casein and tankage gave
poor growth and no protection against selenium poisoning Linseed meal gave
good protection in dogs and was the only protein to protect both rats and dogs
The species differences shown by this work with rats and dogs emphasizes the
need for experimental work with farm animals before any practical applications
can be recommended
   It may be well to mention that although Smith and Stohlman (157) found good
protective action by the proteins of wheat and corn for the rat, experiments with
dogs in this laboratory have shown no particular protective action for these
proteins (104) In fact, the most severe cases of poisoning in dogs have occurred
on rations containing large amounts of these proteins.
   There is disagreement concerning the effect of the fat content of the ration on
selenium poisoning Moxon (103) reported that in a ration containing 37.5
p p m of selenium the percentage of fat in the ration had very little influence on
the toxicity of selenium. Smith (153) observed some beneficial effect from a
ration of low protein and high fat content, but suggests the result may come from
the protein sparing action of the fat.

Effect of other elements   In experiments designed to show the effect of certain other elements administered concurrently with selenium (104) (110) (164) it has been noted that fluorine, molybdenum, chromium, vanadium, cadmium, zinc, cobalt, nickel and uranium caused an increase in mortality   All these elements were given in the drinking water at a concentration of 5 p p m with a ration containing 11 p p m selenium   The same amount of soluble tungsten prevented, to a limited extent, the typical liver lesions caused by selenium and decreased mortality   A level of 2 5 p p m tungsten reduced mortality slightly but did not prevent liver lesions

Surprisingly enough, 5 p p m of arsenic as sodium arsenite administered in the same manner completely prevented the symptoms of selenium poisoning Greater feed consumption and better growth resulted from giving 2 5 p p m of arsenic, but liver lesions were not entirely prevented   DuBois, Moxon and Olson (30) have further shown that sodium arsenite and arsenate are equally effective in counteracting the effect of selenium in seleniferous wheat, while arsenic sulfides (AsS_2 and AsS_3) are ineffective   Sodium arsenite was equally effective against selenium in the form of seleniferous wheat, sodium selenite and the selenium analogue of cystine   It was noted that 10 p p m arsenic in the ration gave slightly better results than 5 p p m in the drinking water, due to a slightly higher arsenic intake   Arsenic was effective in treating rats that had been fed the seleniferous ration for 20 days previous to beginning the arsenic treatment, but was ineffective after the rats had been on the toxic ration for 30 days

Lead had no effect on the toxicity of selenium, but bismuth (subnitrate) had a slightly beneficial effect

It has been noted that growing chicks are more sensitive to arsenic than rats or dogs, and the arsenic treatment of chicks is thus less successful than the treatment of rats or dogs (108)

Subsequent work with chickens (108) has shown that 10 p p m of arsenic in the drinking water nearly counteracts the effect of 14 p p m of selenium in the ration   More recent studies (164) have shown that arsenic markedly improves hatchability of eggs from hens fed a seleniferous ration

Moxon (107) has reported prevention by arsenic of selenium poisoning in a pig raised under laboratory conditions, and Rhian and Moxon (144) have shown that 5 p p m of arsenic in the drinking water will prevent selenium poisoning in dogs fed a ration containing approximately 10 p p m selenium

Recent work in the reviewers' laboratory (164) has further substantiated the relationship of the time of administration of selenium and arsenic noted by DuBois, Moxon, and Olson (30)   When an amount of selenium equivalent to that consumed in a ration containing 12 p p m selenium was injected daily and 5 p p.m of arsenic in the water was given ad lib , death resulted in over half the animals   When the selenium was given in the ration and arsenic equivalent of that consumed in the water was injected daily, no deaths resulted   When the selenium was in the ration and arsenic in the water, no deaths resulted

Arsenic in the form of certain organic compounds (neoarsphenamine and sulfarsphenamine) equivalent to 5 p p m. arsenic in the drinking water, was not quite as effective as sodium arsenite at the same level of arsenic.

It has been noted that antimony trichloride fed in the ration has about the same activity as organic arsenic in preventing selenium poisoning Sodium antimoniate has given no protection against selenium poisoning

Effect of certain organic compounds Stehol (166) showed that bromobenzene and naphthalene were detoxified in the body of the rat, dog and rabbit by conjugation with tissue cystine and methionine and excreted in the urine as *p*-bromophenyl mercapturic acid The close relationship between sulfur and selenium compounds suggested an investigation of the action of bromobenzene and similar compounds in selenium poisoning Moxon et al (118) reported that administration of bromobenzene to rats and dogs fed a seleniferous ration increased the urinary selenium output. Moxon (108) noted that administration of either bromobenzene, benzene or naphthalene to steers grazing on seleniferous range caused a rise in urinary selenium output The selenium content of the blood decreased in most cases This observation suggests that these aromatic compounds aid in converting tissue selenium into a compound that is readily excreted from the kidney

These observations suggest that much more work should be done on the nature of compounds that will promote excretion of selenium from the animal body

Effect of selenium on enzyme systems That selenium has direct effect on certain unicellular organisms and enzyme systems has been known for many years Czapek and Weil (27) state that Chabrie (22) had found sodium selenite to inhibit alcoholic fermentation They concluded that the growing yeast cells reduced the selenite to metallic selenium This inhibiting action was later confirmed by Woodruff and Gies (195) and more recently by Moxon and Franke (112) who extended the observation to show the relative toxicity of selenium and other metals to fermentation systems They found that during yeast fermentation of glucose, as measured by CO_2 production, selenium was more toxic than vanadium, arsenic, and tellurium The toxicity of sodium selenite, selenide and selenate decreased in that order Sodium sulfide partially counteracted the toxic effects of the selenium salts, but elemental sulfur had almost no action of this type. Sodium sulfite, ammonium sulfate and sodium thiosulfate were unable to counteract the toxic effects of selenium Later Lardy and Moxon (81) noted that 0.2 mgm. of arsenic would almost completely counteract the effect of 1 mgm of selenium in inhibiting the fermentation of glucose by bakers' yeast.

Gastler (53) found that selenium salts had no effect on the saccharogenic activity of malt diastase, taka diastase and amylopsin

Levine (88) has reviewed the literature pertaining to the action of selenium salts on bacterial growth and the action of such cultures in reducing selenium compounds.

The action of selenium salts on isolated enzyme systems has been investigated

for various purposes   Collett (23) showed that selenite and selenate inhibit the action of succinoxidase, and in later work Collett and co-workers (24) (25) have used selenium to determine the specificity of certain dehydrogenases

Labes and Krebs (76) found that 0 02 per cent sodium selenite inhibited the action of succinoxidase of pig's muscle   Potter and Elvehjem (142) demonstrated that by using sugars as substrates, selenium caused 80 per cent inhibition in the oxygen uptake of living yeast cells   Less than 10 per cent inhibition was noted when pyruvate or lactate was used as substrate   These workers suggest the action of the selenite may be indirect, through its action on glutathione as has been suggested by Bersin (13)

Wright (191) showed that selenium inhibits the oxygen consumption of mammalian (rat) tissue slices   Using glucose or succinic, pyruvic, lactic or citric acids as substrate, kidney, brain, muscle and tumor slices were incapable of oxidation   With the liver slices the action of selenium was an initial stimulation followed by inhibition   All the tissues rapidly oxidize p-phenylene-diamine in the presence of selenium   Reduced glutathione or pyruvic acid would protect the tissue from the action of selenium if they were added with the selenium   If the selenium was allowed to act for a time before the glutathione or pyruvic acid was added, the oxygen consumption was not restored   Wright concluded that selenium is a general hydrogenase inhibitor   Later Wright (192) measured the effect of diselenodiacetic acid on tissue slices   The selenium in this compound inhibited the oxygen consumption of slices of rat liver, brain, and kidney, but had no effect on muscle tissue   Anaerobic glycolysis of liver, kidney, or tumor slices was not affected by this organic compound

While investigating the effect of selenium on rat livers Wright (193) found that selenium as sodium selenite, selenate or diselenodiacetic acid inhibits the activity of urease but has no specific effect on liver arginase   This further indicates a relationship between selenium and those enzymes dependent on the action of sulfhydryl groups

Bernheim and Klein (12) have classified enzymes on the basis of their reaction to or with selenium into three groups "those relatively insusceptible to selenium, including the glucose, lactate, and pyruvate oxidases of brain and probably l-tyrosine, xanthine, and alcohol oxidases of liver, (b) those in which selenium catalyzes the destruction of an active group, including succinoxidases, choline oxidase, d-proline oxidase, and tyramine oxidase, and (c) l-proline oxidase which is inactivated immediately", suggesting that selenium combines with an active group"

It seems that work on the specific action of selenium on the enzyme systems of the animal body is in its infancy, and much more information is required before the exact mechanism of selenium poisoning can be elucidated

Organic selenium compounds   Painter has reviewed the literature on organic selenium compounds up to 1941 (129) so only those compounds of immediate biological interest will be discussed here

The early work of Franke (37) and Franke and Painter (41) (42) (131) (133) (130) showed definitely that the selenium in toxic cereals was associated with the

proteins of the cereals   The studies indicated that the selenium is an integral part of the protein molecule and consequently there has been much speculation regarding the possibility of selenium replacing sulfur in cystine and methionine The selenium analogue of cystine has been prepared by Fredga (51) (52) and Painter (135)   Painter (135) has also recently prepared phenyl-seleno-cysteine, benzyl-seleno-cysteine, benzyl-seleno-homocystine
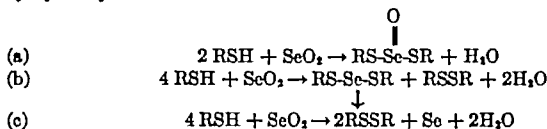
Painter, Franke and Gortner (134) synthesized organic diselenides, selenium ethers and seleninic acids of acetic, β propionic, n propyl, and benzyl radicals They studied the relationship of these compounds and the selenium compounds in plants (133) in regard to their decomposition in alkaline solutions   Moxon, Anderson and Painter (109) have compared the toxicities of these organic compounds and selenium in wheat.

The toxicity of the selenium analogue of cystine (106) (111) is comparable to the toxicity of selenium in seleniferous grains and in sodium selenite, while toxicity of selenium in all other organic selenium compounds investigated has been much less (106) (109) (111)   This is further evidence that selenium may occur in cereal proteins as the selenium analogue of a sulfur amino acid   Horn and Jones (62) have isolated a crystalline amino acid complex containing selenium and sulfur from highly seleniferous *Astragalus pectinatus*   They have tentatively assigned a structure to the components of their complex and they state "It is believed that the selenium in toxic wheat and other grains is combined in the protein as an amino acid having a similar structure "

They have not published data on the toxicity of the amino acid complex but Horn, Jones, and Ringle (63) have published articles on the isolation of a new sulfur-containing amino acid (lanthionine) from sodium carbonate-treated wool Lanthionine is similar in structure to the selenium containing amino acid complex. Du Vigneaud and Brown (179) have synthesized lanthionine so it should be possible to synthesize the selenium analogue and determine its toxicity

There is very little definite information on the mechanism of selenium toxicity There is however, some information on its effect on unicellular organisms and enzyme systems (see section on Effect of Selenium on Enzymes)

Selenite will oxidize sulfhydryl compounds forming disulfide and an unstable RS-Se-SR compound   According to Painter (129) the action of selenite on sulfhydryls may take three courses

(a) $$2\ RSH + SeO_2 \rightarrow RS\text{-}\overset{\displaystyle O}{\overset{\displaystyle \|}{Se}}\text{-}SR + H_2O$$

(b) $$4\ RSH + SeO_2 \rightarrow RS\text{-}Se\text{-}SR + RSSR + 2H_2O$$
$$\downarrow$$
(c) $$4\ RSH + SeO_2 \rightarrow 2RSSR + Se + 2H_2O$$

Stekol (167) has reported the preparation of selenium tetracysteine from sodium selenite and cysteine hydrochloride   Using the same compounds Painter (129) reported the preparation of selenium dicysteine as shown below

$$\underset{\displaystyle NH_2}{HOOC\text{—}CH}\text{—}CH_2S\text{—}Se\text{—}SCH_2\text{—}\underset{\displaystyle NH_2}{CHCOOH}$$
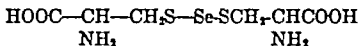
Compounds of the type RS—Se—SR are unstable as shown by reactions (b) and (c) above and are, therefore, difficult to isolate in the pure form   Painter (135) is of the opinion that the reaction of selenite and cysteine would take place according to reactions (b) and (c) rather than by the reaction which Stekol has reported

Bersin (13) prepared HOOCCH₂S—Se—SCH₂COOH from thioglycolic acid and selenite   He believes that a similar unstable compound is formed from selenite and glutathione   Crude glutathione isolated from the blood of selenized cattle contains some selenium (2)   DuBois, Rhian and Moxon (31) have shown that injections of reduced glutathione will protect rats against doses of selenite which ordinarily cause death   The administration of arsenic (either oral or injected) will likewise protect rats against the toxic action of selenium (either oral or injected)   This action of arsenic would suggest the possibility of some reaction between arsenic and selenium compounds *in vivo*   Arsenious acid will react with cysteine to form arsenious tri-cysteine (71) (167)   Possibly arsenic reacts with selenium compounds in such a way as to keep the selenium compounds from attacking the sulfhydryl compounds of the body

Public Health   The relationship of selenium poisoning to public health was reviewed by Manville (93) in 1938 and by Smith in 1940 (154)

Smith, Franke and Westfall (155) in 1935 made a survey involving 111 families living in seleniferous areas of Wyoming, South Dakota, and Nebraska to determine the possibility of selenium intoxication through the ingestion of seleniferous foodstuffs   They found that urines from 92 per cent of the individuals examined contained selenium in amounts varying from 2 to 133 micrograms per 100 cc   This, of course, gave definite proof that selenium was being ingested, but they were unable to discover any symptoms pathognomonic of selenium poisoning   Smith and Westfall (160) made field studies in 1936 and were unable to reach any more definite conclusions about the symptomatology of selenium poisoning in humans

Lemley (85) and Lemley and Merryman (86) became interested in selenium poisoning in humans in connection with several cases of acute dermatitis   Urine specimens from these cases contained selenium in amounts comparable to the selenium content of urines collected from farm animals suffering from selenium poisoning   Lemley and Merryman (85) (86) administered bromobenzene to the patients with dermatitis and found that it gave immediate relief, and most cases were cleared completely within two or three weeks   Westfall and Smith (184) were unable to increase the rate of elimination of selenium from rabbits by the administration of bromobenzene and have discouraged its use in human cases of selenium poisoning   Nevertheless, it is still being used by physicians in seleniferous areas who report that it is the only treatment which will clear up the particular dermatitis which appears to be associated with selenium

More field and clinical investigations need to be carried out in the seleniferous areas in an effort to establish the symptomatology of selenium poisoning in humans and to arrive at some definite tolerance levels for selenium in foodstuffs

Dudley (33) has discussed the hazard of selenium poisoning among workers in

industrial operations where selenium is handled   Both Dudley (33) and Hamil ton (59) have described the symptoms of human cases of selenium poisoning caused by the inhalation of volatile selenium compounds   Halter (58) reported a human case of industrial selenium poisoning   Painter (129) has described his experiences in connection with the accidental inhalation of concentrated hydrogen selenide

Motley, Ellis and Ellis (102) reported experiencing sore throats after working with sodium selenite and after handling animals which had been injected with sodium selenite   The reviewers, however, have never experienced sore throats at any time which could be attributed to the handling of selenium compounds or handling of animals which had been injected with selenium compounds

Byers (16) discovered some cases of selenium poisoning in humans in Mexico which resulted from soils being contaminated by selenium in the wastes from silver and gold mines

Sterner and Lidfeldt (168) have determined the selenium content of urine samples from what they call normal subjects   They find more selenium than should be present unless the subjects are exposed to some source of selenium Selenium has been investigated at different times as a possible cure for cancer A few typical references on this work are (28) (72) (149) (181) (182)

Methods of analysis for selenium   Painter (129) has reviewed the methods of analysis for selenium   Most of the selenium determinations which have been made on rocks, soils, and plant and animal tissues have been made by the methods of Robinson et al (148) and Beath, Eppson and Gilbert (8)   During the past two years the reviewers have used the A. O. A. C. method as described by Klein (73)   This method is especially accurate for small quantities of selenium

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BIOCHEMICAL PROBLEMS OF THE CHEMO-AUTOTROPHIC BACTERIA

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1 *Development of the concept of chemo-autotrophism*   When Winogradsky came to de Bary's laboratory, he undertook to study the then vital problem of the alleged pleomorphism of the "sulfur bacteria "   For an exact investigation pure cultures were desirable, if not imperative, and it was undoubtedly anticipated that with such cultures it would be an easy matter to conquer the last important stronghold from which the doctrine of bacterial pleomorphism could still be defended   But the early attempts at growing the organisms in laboratory media led to developments in the field of physiology whose significance overshadows the final demonstration that the putative morphological variability exists no more in this group than among other bacterial species   Careful observations and deductions, supported by a few simple, conclusive experiments, led the young scientist to the revolutionary concept that certain micro-organisms, although devoid of chlorophyll, can nevertheless maintain themselves, and even develop, in the absence of organic substances

Typical for the natural habitat of the sulfur bacteria is the invariable presence of hydrogen sulfide, the organisms themselves are found stuffed with sulfur globules   The correlation had given rise to interpretations involving a causal relationship, it being held that the sulfide was produced from sulfur by the sulfur bacteria   Winogradsky's earliest experiments, however, showed convincingly that this could not be so   And, struck by the gradual disappearance of the sulfur globules from cells which were kept for some days in various culture solutions, he supplied additional hydrogen sulfide to the cultures, whereupon sulfur droplets soon reappeared in the organisms   From these observations Winogradsky conceived the causal relation to be otherwise   The bacteria would be restricted to a sulfide-containing environment because they need this substance which they oxidize to sulfur, the oxidation process furnishing the catabolic energy which the more common organisms derive from the oxidation or fermentation of organic materials   Experiments showed that, when sulfide is not available, the sulfur bacteria can maintain themselves by oxidizing the stored sulfur to sulfate

The studies on the sulfur bacteria (1, 2) were soon followed by similar investigations on iron bacteria (3), occurring normally in water with ferrous salts in solution   The deposition of ferric hydroxide was considered as due to a comparable physiological oxidation process

The full consequences of the idea that the oxidation of an inorganic substance could provide the energy for vital processes were realized when Winogradsky succeeded in isolating the nitrifying bacteria in pure culture   With these it was shown that the oxidation of ammonia to nitrite, and of nitrite to nitrate, enables the respective organisms to synthesize their cell materials entirely from carbon

dioxide and minerals in darkness (4)   This process of carbon dioxide assimila tion has been designated by Pfeffer (5) as chemosynthesis, and the organisms as chemosynthetic   More recently the better term chemo-autotrophic has been proposed (6)

Amply corroborated by subsequent workers, the existence of chemo-auto trophic micro-organisms thus became established, and various species have since been isolated which can live and grow in strictly mineral media   The chemical energy derived from the oxidation of sulfide, sulfur, sulfite and thiosulfate, of comparable selenium compounds, of ammonia and nitrite, or of molecular hydro gen, can adequately be substituted for the radiant energy which permits green plants to synthesize organic matter in the light

Such processes are not restricted to aerobic conditions   Oxidation of sulfur and thiosulfate with the simultaneous reduction of nitrate allows the develop ment of *Thiobacillus denitrificans* in the absence of air (7, 8, 9), similarly certain hydrogen bacteria can grow anaerobically in the presence of nitrate (10)   In addition to these chemo-autotrophic processes of nitrate reduction sulfate reduc tion with molecular hydrogen is also known (10, 11, 12, 13) although decisive experiments to show development of the sulfate reducing bacteria in mineral media, on the basis of oxidation of hydrogen with sulfate, have not yet been carried out   Even in the absence of oxygen, nitrate, or sulfate, hydrogen gas may lead to bacterial development, as during the conversion of hydrogen-carbon dioxide mixtures to methane (14) or acetic acid (15)

In general, it can therefore be said that chemical reactions between inorganic substances, which take place with an increase in entropy, can be used by micro organisms as a source of energy with which the synthesis of organic matter from carbon dioxide is possible

The early studies with pure cultures of chemo-autotrophic bacteria led to another discovery which emphasized the physiological peculiarity of these organisms   It was again Winogradsky who established that the nitrifying bac teria are adversely affected by the presence of organic compounds, low concen trations inhibiting growth, higher ones even preventing their metabolic activi ties.   Comparable results were later reported for some of the sulfur bacteria (8, 16)   The inability of nitrifying and sulfur-oxidizing bacteria to utilize organic substances has often been contested, but the more careful investigations of this problem (17, 18, 19) have sustained Winogradsky's claims

Nevertheless, it has been recognized from the beginning that the hydrogen bacteria can all be grown equally well in the absence of hydrogen, at the expense of organic matter   Also certain *Thiobacillus* species can develop normally in organic media even in the absence of oxidizable sulfur compounds (8, 19 20, 21) While these findings made it impossible to apply Winogradsky's rigorous defini tion of the physiological characteristics of ' inorgoxidants" (22)—which included the inability to decompose organic substances—to all chemo-autotrophic bac teria, they did not present a serious obstacle to the formulation of a clear-cut criterion for the latter   As such could be considered any organism capable of development in darkness in a strictly mineral medium, by the oxidation of an

inorganic substance, and the concomitant assimilation of carbon dioxide   This definition eliminated the inclusion of various organisms which, like *Aspergillus niger*, can oxidize elementary sulfur, but only in organic media (23)   Oxidations of this type appear more nearly comparable with phytochemical reductions, they represent a side-reaction of doubtful physiological significance rather than the primary source of energy for the organism concerned

Recent advances have, however, made this definition also rather illusory as a satisfactory and unequivocal means of distinction between chemo-autotrophic and heterotrophic organisms   A good example of the difficulty is furnished by the hydrogen bacteria   Already Niklewski (10) made the observation that either one of two pure cultures, isolated from profusely growing crude cultures, did not grow by itself in the mineral medium, whereas inoculation of the two types together resulted in excellent growth   From unpublished experiments of Dr M Doudoroff (personal communication) the explanation of such a "symbiotic" relationship appears to be that each type of these hydrogen bacteria requires one or more organic growth factors, produced in excess by the other kind, a situation very similar to the "symbiosis" of certain yeasts and molds which will grow in a mineral medium with sugar when inoculated together, although neither one appears capable of such development by itself (24)   This has been shown to be due to the fact that one of the symbionts needs the pyrimidine part of the thiamin molecule for growth, which is produced in excess by the other partner, while the latter requires the thiazole moiety, furnished in turn by the former

In the case of the hydrogen bacteria it is evident that the combination of the two types can be designated as chemo-autotrophic, either one alone does not correspond to the definition   Although the growth factors for the hydrogen bacteria have not yet been worked out, each one of the two species can be grown in pure culture, and largely by oxidizing hydrogen and reducing carbon dioxide, provided that the medium is supplemented with a small amount of organic matter, the latter serving as a source of supply of one or more substances which the organism needs in a small quantity but which it is unable to synthesize   The question then arises whether this quantitatively insignificant lack of synthetic ability should be regarded as a sufficient reason for excluding the species from the group of chemo-autotrophic bacteria, thus losing sight of its major physiological characteristic, viz , that of being able to synthesize the bulk of its cellular constituents from carbon dioxide with energy derived from the oxidation of an inorganic substance

It would, of course, be possible to re-define chemo-autotrophism in such a manner that the above difficulty could be avoided, for example by emphasizing the occurrence of a carbon dioxide assimilation in the dark   But at present such attempts also appear doomed to failure   This is due to the discoveries of the past 7 or 8 years that carbon dioxide assimilation or reduction is far from limited to organisms which one might still be inclined to accept, though not in the strictest sense of the word, as chemo-autotrophs   It has been shown that *E coli* can convert carbon dioxide and hydrogen into formic acid (25), that the

bacterial production of methane is actually a process of complete carbon dioxide reduction (26, 27), that propionic acid bacteria (28 29), E $coli$ (30), molds (31), and even protozoa (32, 33) and animal tissues (34, 35) can assimilate carbon dioxide   In the last-mentioned cases the assimilated carbon occurs normally in the form of acidic metabolic products, among which the four-carbon dicarbo-xylic acids are the most important (36, 37)   In spite of this genuine carbon dioxide assimilation no one would be willing to apply the epithet chemo-autotrophic to the organisms in question, whose metabolism, under all circumstances, consists predominantly of decomposition processes of organic substances

At one time the differentiation between an autotrophic and heterotrophic mode of life carried the implication that the former was quite independent of the activities of other living organisms   In a measure this, of course, is true.   But considering what the reality of the cycle of matter connotes, this viewpoint is also vulnerable, ultimately the autotrophs now living are as much dependent upon the heterotrophs as $vice$ $versa$ And fortunately, the important problem is not so much to find an unassailable definition of chemo-autotrophism as it is to reach a better understanding of natural phenomena   Hence we may leave the decision as to whether the above-mentioned hydrogen bacteria and various other organisms with similar properties should properly be called chemo-autotrophs or heterotrophs to those who can make out a good case for its significance   This attitude seems the more justifiable because a number of other facts have tended to cause a merging of the two designations

2 $Energetic$ $relations$ in the $metabolism$ of $chemo$-$autotrophic$ $organisms$ The great importance of the chemo-autotrophic mode of life lies in the fact that it admits of a comparative study of the biochemistry of carbon dioxide assimila tion   So long as this process was considered as the special prerogative of green plants, it was necessary to regard the photochemical nature of the reaction as essential for the fate of carbon dioxide   It could, therefore, not be decided whether carbon dioxide assimilation was connected with the absorption of radiant energy in a direct or indirect manner   The existence of organisms which could accomplish an assimilation of carbon dioxide in the dark showed conclusively that for the conversion of carbon dioxide into organic matter the absorption of light was not a necessary prerequisite

In looking for a common denominator of the photo- and chemo-autotrophic assimilation processes, it became clear that a meeting ground could be found by considering the energetic relations   In both cases one could point to a source of energy by which the synthesis of organic substances from carbon dioxide was made possible   To the photosynthetic organisms the energy was obviously supplied by absorbed light quanta, while to the colorless chemo autotrophs it was furnished by an exergonic chemical reaction [1]

Thus it becomes understandable that studies on the energy relations were among the first which were carried out with chemo-autotrophic bacteria   Ini tially such investigations dealt with a determination of the quantity of inorganic

[1] The term 'exergonic' is used in preference to exothermic   See C D Coryell Sci ence 92 380 1940

substrate that was oxidized and of the amount of organic matter meanwhile synthesized by the organisms   The application of thermodynamic data to the results then gave rise to computations of the "efficiency" of the processes, and to a comparison with that of the photosynthetic reaction   Since this particular phase has been reviewed in a previous volume of this Journal (38), it will suffice to summarize the results   As a rule, the efficiency of a variety of types of chemo-autotrophic metabolism was found to be of the same order of magnitude ($\pm$ 7 per cent)   The hydrogen bacteria, as an exception, appeared noticeably more efficient, from Ruhland's data (39) on the ratio of hydrogen oxidized to carbon dioxide assimilated an efficiency of over 25 per cent was calculated.   It was further stressed that none of the chemo-autotrophic processes even approaches the efficiency of photosynthesis which, according to the results of Warburg and Negelein and of Wurmser, is around 80 per cent

In the light of recent studies of the photosynthetic reaction this comparison can no longer be considered valid, the high efficiency values have not been substantiated   First, Arnold (40) and Manning et al (41), using different methods of experimentation, arrived at considerably lower values ($\pm$ 35 per cent)   The discrepancy with the earlier data has been satisfactorily accounted for by the fine work of Emerson and Lewis (42), in which the manometric measurements of Warburg and Negelein could be fully corroborated, but which also showed that the method of computation of the efficiency was incorrect because the underlying assumption that the gas produced during brief periods of illumination is exclusively oxygen is contrary to the facts   At present, therefore, the best values for the efficiency of photosynthesis must be accepted as well below 40 per cent

Secondly, there is a good reason for maintaining that this high efficiency measured during photosynthesis is not really comparable with the figures computed by Becking and Parks for chemo-autotrophic processes   The former is based upon metabolism studies of short duration so that one may reasonably expect that the carbon dioxide assimilation has resulted chiefly in the formation of the primary assimilation product, carbohydrate   In addition, the assimilation values are always corrected for the simultaneously occurring respiration   The data on chemo-autotrophic organisms, on the other hand, are derived from experiments in which the extent of carbon dioxide assimilation was determined by measuring the organic carbon content of cultures in mineral media after a period of growth of the bacteria often extending over weeks   Consequently the assimilation process must have gone far beyond a primary assimilatory product, all the different cell constituents, ultimately derived from the primary product by secondary reactions, are included in the final determination   It will be evident that such secondary processes, as, for example, the formation of lipids, may involve quite considerable losses   Furthermore, corrections for possible respiration processes in which organic compounds are oxidized have not been applied here   Thus it becomes clear that the measured or computed efficiencies in those cases must fall considerably below the maximum values which would represent the

efficiency of the primary carbon dioxide assimilation in a chemo autotrophic process. The above-mentioned attempts at evaluating the efficiency of chemo-autotrophs have, therefore, not contributed much beyond the demonstration that the over all conversion of carbon dioxide into the various components of bacterial cells is less efficient than the production of a primary assimilate from carbon dioxide by photosynthesis.

While Ruhland was fully aware that his data did not give an accurate idea of the true efficiency of the carbon dioxide assimilation of hydrogen bacteria and left it at that,[2] Burk (43) believed that in this case an accurate computation of the 'thermodynamic efficiency'—as contrasted with Ruhland's "machine efficiency"—could be accomplished by applying to the over all figures a correction for energy consumption not directly connected with the assimilation process. In this manner the conclusion was reached that this assimilation must be considered as, thermodynamically, completely reversible. On account of its theoretical importance this paper will be considered in some detail, especially because the method of calculation rests upon an assumption which is open to serious doubt.

Ruhland had shown that the hydrogen bacterium, *Bacillus pycnoticus*, when growing in the presence of both glucose and hydrogen, does not appear to oxidize the latter. From this and similar observations Burk drew the inference that the oxygen consumption by these hydrogen bacteria should be ascribed in its entirety to an oxidation process of organic substances, and that oxygen is not used at all for the direct oxidation of molecular hydrogen. Starting with this assumption it then follows that the oxygen utilization in Ruhland's experiments can only be accounted for by an oxidation of organic compounds previously synthesized from carbon dioxide and hydrogen. This would permit of a calculation of the total organic matter synthesized by the bacteria during the experiments by adding the equivalent of the oxygen consumed to the amount formed during growth in a culture in inorganic media. If this is done, the total carbon dioxide assimilation corresponds very closely to that required by the reaction equation

$$CO_2 + 2H_2 \rightarrow \tfrac{1}{6}C_6H_{12}O_6 + H_2O$$
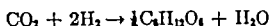
the slight deviation being due to the fact that the average cell material of the bacteria is slightly more reduced than carbohydrate.

Burk further showed that, since the oxidation of 2 mols of hydrogen can furnish 108460 cal of free energy, and the reduction of 1 mol of carbon dioxide

requires 105140 cal, the theoretical relation for the above process, if it were completely reversible, is

$$\frac{\text{No of mols } H_2 \text{ needed}}{2 \times \text{No of mols } CO_2 \text{ reduced}} = \frac{105140}{108460} = 0\ 970$$
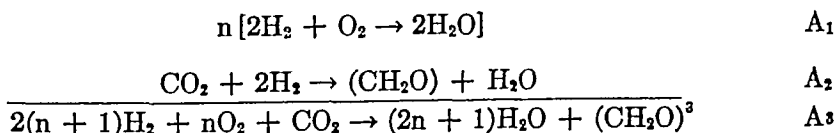
The experimental data, treated in the above manner, furnish practically the same result.

$$\frac{\text{No of mols } H_2 \text{ used}}{2 \times \text{No of mols } CO_2 \text{ reduced}} = 0\ 966 \pm 0\ 012,$$
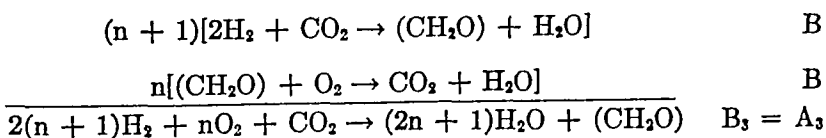
from which follows the conclusion that the reduction of carbon dioxide is indeed a perfectly reversible process

This argument seems, however, fundamentally deficient, and amounts to proving an assumption by taking its correctness for granted, because in the original assumption the outcome of the computation is already implied   This follows from a simple analysis

The culture of hydrogen bacteria growing in a mineral medium in the presence of hydrogen contains as oxidizable materials only hydrogen and the organic matter of the bacterial cells   The consumption of oxygen must, therefore, be due to an oxidation of A, molecular hydrogen, B, organic matter, or C, both Since meanwhile organic matter is formed from carbon dioxide, case A can be represented by the set of equations

$$n\,[2H_2 + O_2 \rightarrow 2H_2O] \qquad\qquad A_1$$

$$\frac{CO_2 + 2H_2 \rightarrow (CH_2O) + H_2O}{2(n + 1)H_2 + nO_2 + CO_2 \rightarrow (2n + 1)H_2O + (CH_2O)^3} \qquad \begin{matrix} A_2 \\ \\ A_3 \end{matrix}$$

Similarly, case B corresponds to

$$(n + 1)[2H_2 + CO_2 \rightarrow (CH_2O) + H_2O] \qquad\qquad B$$

$$\frac{n[(CH_2O) + O_2 \rightarrow CO_2 + H_2O]}{2(n + 1)H_2 + nO_2 + CO_2 \rightarrow (2n + 1)H_2O + (CH_2O)} \qquad \begin{matrix} B \\ \\ B_3 = A_3 \end{matrix}$$

while case C is a combination of A and B

The analytical data concerning hydrogen, oxygen and carbon dioxide utilization and organic matter production in Ruhland's experiments obviously apply only to the final, overall equations   And, since these are identical for all three cases, a decision as to which reactions occur must be based upon other considerations   Now, the assumption that all oxygen, consumed during the growth of the organisms, has been used for the oxidation of (CH_2O) implies that the hydrogen can only have reacted according to equation B_1 That a computation on this premise leads to the theoretical value of the entropy relations is,

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[3] In these equations (CH_2O) designates the assimilation product, not formaldehyde Though an approximation, it is sufficiently accurate for the purpose, and has the advantage of simplicity

therefore, obvious Burk s results could thus have been anticipated But they prove nothing beyond the fact that Ruhland's analyses have been accurate

It has already been mentioned that all known hydrogen bacteria can live heterotrophically As such they are capable of oxidizing a great variety of organic compounds It could therefore, be expected that in the presence of both hydrogen and an organic substrate the oxidation of the former is suppressed Furthermore, the inability of bacteria to oxidize hydrogen when grown in organic media is often due to selection, adaptation, or both Hence the observations of Ruhland on the inhibition of hydrogen oxidation by glucose do not justify the assumption that autotrophically growing hydrogen bacteria use oxygen only for the oxidation of organic substrates

In three recent reports (44, 45, 46) on the oxidation of molecular hydrogen by hydrogen bacteria, purple bacteria, and green algae it has been pointed out that in all cases this reaction is dependent upon the presence of carbon dioxide This finding may seem to strengthen Burk's assumption which would naturally require the presence of carbon dioxide for the utilization of hydrogen It must, however, be borne in mind that also for the oxidation of organic substances the presence of carbon dioxide is a prerequisite (47) and here the explanation for this phenomenon must evidently be quite different (37) Thus it is readily conceivable that the reason for the necessity of carbon dioxide in the oxidation of hydrogen would be the same as in the oxidation of organic compounds

3 Biochemical mechanisms in the chemo-autotrophic bacteria As long as the link between chemo-autotrophic and photosynthetic carbon dioxide assimilation was considered to be principally an energetic one, the approach outlined in the previous section was the most promising It could have been refined by attempts at obtaining a clearer separation of the primary assimilation process from secondary conversions, for example by conducting experiments of short duration In addition, careful measurements of an endogenous respiration process, both before and after a period in which an inorganic substrate was being oxidized, might have permitted the application of a satisfactory correction for the destruction of organic matter during the assimilation process proper Such refinements would have yielded data on the efficiency of the assimilation more directly comparable with those available for photosynthesis However, the interest in a comparative study of the two assimilation processes had mean while begun to move in a different direction

Where energy from absorbed light quanta or from exergonic chemical reactions is used for the conversion of carbon dioxide into organic matter, there must exist a mechanism for the transfer of energy The gradual development of a better understanding of the chemical mechanisms involved in catabolic processes both oxidative and fermentative, had been spectacular, and toward the end of the 1920 s a clear enunciation of the major principles of these mechanisms had been achieved Briefly these principles can be summarized as follows

1 Any biochemical process can be considered as consisting of a series of individual step-reactions

2 Each one step constitutes a chemically intelligible and extremely simple type of reaction

3 The common property of all such reactions is that they can be regarded as examples of hydrogen (electron) transference from one constituent to another

4 Thermodynamically each step is a spontaneous (exergonic) reaction

The most general expression of these principles was given in Kluyver's well known reaction equations (48)

$$AH + B \rightarrow A + BH$$
$$AH\ B \rightarrow A\ BH$$
$$AH\ B \rightarrow A + BH$$
$$AH + B \rightarrow A\ BH$$
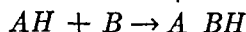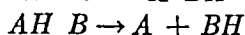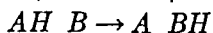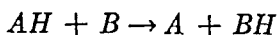
It is understandable that the fruitfulness of these concepts of the intimate mechanism of catabolic processes led to a desire to apply them to those bio-chemical reactions which are known as anabolic   Since the conversion of carbon dioxide into organic matter is quantitatively and economically the most important instance of the latter, early attempts were made to formulate possible step-reactions for this process which would be in harmony with these ideas

Photosynthesis soon appeared amenable to such an interpretation, at least in part, when it was found that certain colored bacteria, the green and purple sulfur bacteria, carry out a reduction of carbon dioxide which is dependent upon both illumination and the presence of oxidizable substances, and which proceeds without the evolution of molecular oxygen (49, 50)   These results led to a generalized formulation of the photosynthetic process as rendered by the equation

$$CO_2 + 2H_2A \xrightarrow{\text{Light}} (CH_2O) + H_2O + 2A$$
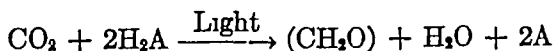
From this equation it would appear that green plant photosynthesis represents a special case in which H_2O serves as the hydrogen donor, H_2A, which is dehydrogenated to molecular oxygen

It thus became possible to compare the features of various types of carbon dioxide reduction in the hope of thereby establishing those characteristics which are common to all such processes, and of eliminating secondary, perhaps fortuitous aspects   An approach of this kind, for which Kluyver had coined the name "comparative biochemistry" (48), has frequently yielded remarkably instructive results (see, $e\,g$, (51))

Such a comparison of the different photosynthetic processes reveals the fact that the common features are the absorption of radiant energy and the reduction of carbon dioxide, while the specific hydrogen donors may differ widely   Since, furthermore, the oxidation of all the possible hydrogen donors, with the sole exception of H_2O, can also be accomplished by the respective organisms in the dark with the utilization of oxygen, it appeared as if there would exist a direct link between the carbon dioxide reduction and the absorption of light quanta

But here the existence of chemo-autotrophic organisms warns to caution, their metabolism shows unambiguously that the reduction of carbon dioxide *per se* does not require light   And because, in this group of organisms, we are therefore compelled to admit the existence of a mechanism whereby the conversion of carbon dioxide into organic matter can be accomplished without the co-opera-

tion of radiant energy, our present concepts of "comparative biochemistry" make it tempting to suppose that the same, or at least a closely similar mechanism, may well be operative in photosynthetic organisms too Some recently established facts make this even more than suggestive
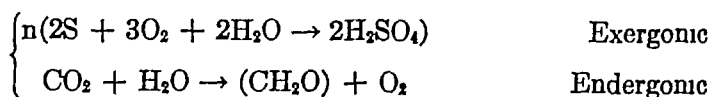
Among the photosynthetic purple bacteria are certain species which can assimilate carbon dioxide in the presence of molecular hydrogen These organisms can also be grown in the dark like regular hydrogen bacteria Instead of carrying out a photosynthetic metabolism they now behave as chemo-autotrophs A similar conversion of photochemical to non photochemical carbon dioxide assimilation has been accomplished with certain green algae by Gaffron (46) These observations force us to the conclusion that either two entirely different mechanisms for carbon dioxide reduction exist side by side in the same organism, or that the biochemical mechanism for the assimilation of this compound is always independent of light in these organisms, and that the absorption of radiant energy in photosynthesis is immediately connected with a step reaction which is somewhat removed from the reduction process itself At present the tendency is distinctly in favor of the latter alternative In this line of thought a study of the metabolism of the chemo-autotrophic bacteria naturally becomes all the more important because it may contribute to an elucidation of the mechanism of carbon dioxide assimilation in photosynthesis as well

Recently some advances of great interest have been made in this direction Experimenting with the colorless sulfur bacterium *Thiobacillus thiooxidans*, Vogler has found that the assimilation of carbon dioxide, which in growing cultures accompanies the oxidation of sulfur to sulfuric acid, can be separated in time from the energy yielding oxidation process A suspension of the organisms, supplied with a small quantity of sulfur in the absence of carbon dioxide, rapidly consumes oxygen until the sulfur has been completely oxidized If at this point carbon dioxide is admitted, a rapid uptake of carbon dioxide is observed It could, moreover, be shown that this assimilation process is entirely independent of simultaneous oxidation reactions because it occurs, and quantitatively to the same extent, in an atmosphere devoid of oxygen (52) From these results it may, therefore, be concluded that during the oxidation of sulfur, and in the absence of carbon dioxide the energy released during the process is stored up in the cells and can be used afterwards for the assimilation of carbon dioxide It is then logical to infer that normally, when the bacteria oxidize sulfur and grow in the presence of carbon dioxide, the assimilation proceeds by the same mechanism

The discovery that oxidation of sulfur and assimilation of carbon dioxide do not necessarily occur simultaneously, but can be separated in time, suggests strongly that the mechanism of energy storage and release involves the building up of a store of chemical substances with a high energy content upon which the cells can draw at some later time in order to make possible the occurrence of a reaction which by itself is endergonic And the very fact that the net result can be represented by a reaction equation in which substances other than sulfur, oxygen, carbon dioxide, and the assimilation product do not occur implies that the 'high-energy compounds' would be involved in a cyclic change It then

becomes possible to formulate the chain of events associated with the chemo-autotrophic metabolism somewhat more satisfactorily

Instead of the more conventional representation

$$\begin{cases} n(2S + 3O_2 + 2H_2O \rightarrow 2H_2SO_4) & \text{Exergonic} \\ CO_2 + H_2O \rightarrow (CH_2O) + O_2 & \text{Endergonic} \end{cases}$$

amplified by the statement that these reactions are energetically coupled, a series of reactions can be postulated as follows

$$n(2S + 3O_2 + 2H_2O + X \rightarrow 2H_2SO_4 + Y)$$

$$CO_2 + nY \rightarrow (CH_2O) + nX$$
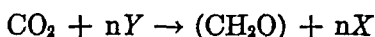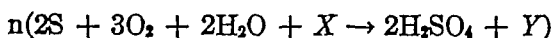
in which both steps are exergonic

At first sight this modified formulation does not appear particularly useful as long as X and Y remain unspecified entities   It might also, and rightly, be objected that it does not present an unusual or new feature   Physical chemists as well as those biologists who have occupied themselves with studies on the actual mechanism of energy transfer have long felt that the energetic coupling of two reactions requires a chemical mediator   And, if no further evidence as to the nature of X and Y were available, it would indeed be futile to stress this formulation   The point to be made is, however, that there are good reasons to believe that in this respect important information has been gained which not only tends to reveal the nature of the chemical mediators, but also indicates the very general application in biochemical processes of one and the same group of compounds in this capacity

Vogler and Umbreit (53) have, namely, succeeded in demonstrating the direct coupling of phosphate transformations with both sulfur oxidation and with carbon dioxide assimilation   Evidently this became possible only after the experimental separation of the last-mentioned reactions had been accomplished   Their studies on the amounts of phosphate derivatives in the medium and in the cells had previously shown that in the combined presence of sulfur, oxygen and carbon dioxide significant changes in the phosphate compounds could not be observed   But if *Thiobacillus thiooxidans* was allowed to oxidize sulfur in the absence of carbon dioxide the oxidation was found to be accompanied by the disappearance of inorganic phosphate from the medium, and its conversion, in the cells, into an organic phosphate ester   Conversely, the subsequent assimilation of carbon dioxide, unaccompanied by a simultaneous oxidation of sulfur, results in the concomitant release of inorganic phosphate to the medium   It thus appears that in the assimilation process of *Thiobac thiooxidans* a phosphate cycle is involved in which the product responsible for the storage and transfer of energy can be identified with an organic phosphate ester   The composition of the organo-phosphates of *Thiobacillus* cells has been elucidated by the recent analyses of Le Page and Umbreit (54), and in a personal communication Umbreit has stated that the energy-storage material has been shown to be adenosine-triphosphate, identical with that from yeast

The significance of this discovery can hardly be over-estimated Not only does it permit of a clearer concept of the nature of the terms X and Y, but it further reveals that the chemical mechanisms for energy transfer are very closely similar in all living organisms, including autotrophic as well as heterotrophic cells Particularly impressive in this connection becomes the "utility in biochemistry," advocated so convincingly by Kluyver and Donker (55) as early as 1926, to which now the generality of the phospho-organic esters as energy transferring compounds may be added The important rôle played by these esters in biological syntheses and in the mechanism of muscular contraction have recently been clarified by the important contributions of Cori et al , Hanes, Kalckar, and especially Lipmann (56–59) It now appears likely that also the initial stages of carbon dioxide assimilation in chemo-autotrophs are associated with these substances And Ruben (60) has just proposed a well-conceived working hypothesis of the mechanism of photosynthesis in which these aspects are incorporated

Two problems have been opened up by this new development. The first concerns a clarification of the reaction steps in which inorganic phosphate becomes esterified, the second, the establishment of the actual mechanism of the initial carbon dioxide fixation Regarding the former point nothing can be said as yet with any degree of certainty In the metabolism of organic compounds phosphate esters arise in which, through dehydrogenation, the phosphate bond energy can become so increased as to represent up to 12 000 calories Whether similar phosphate esters are possible with inorganic sulfur compounds, or whether the latter themselves are combined with organic phosphate esters, conceivably replacing the phosphates, is unknown

Also the chemical reactions by which carbon dioxide is converted into an organic substance have not yet been elucidated, although much progress has been made in this respect during the past few years One of the best established mechanisms is that in which a four-carbon dicarboxylic acid is formed from carbon dioxide and a three-carbon compound, the latter in all probability being pyruvic acid (36, 37, 61, 62) That this mechanism may not be operative in the chemo-autotrophic bacteria seems to follow from the observation of Umbreit et al that here pyruvate in low concentration inhibits carbon dioxide fixation However further experiments are needed, and it seems likely that any advances made in this field will immediately reflect on our present concepts of carbon dioxide assimilation by any one organism, whether photosynthetic, chemo-autotrophic, or heterotrophic It is becoming increasingly evident that all biochemical reactions follow similar general pathways, so that a better understanding of one is soon followed by direct applications to other processes

4 *Autotrophic organisms and the metabolism of organic matter* One aspect of the transformation of carbon dioxide into cellular materials remains to be discussed This concerns the means whereby the great variety of cell constituents are formed Two main possibilities offer themselves as working hypotheses The first is that each substance is produced more or less directly from carbon dioxide and whatever other minerals enter into its composition The alternative

would be the assumption that only one or a very few organic substances result from the process of carbon dioxide assimilation *per se,* and that the different and manifold components which together constitute the cell originate by a series of secondary transformations of such primary assimilation products From the point of view of "comparative biochemistry" the latter hypothesis seems infinitely more probable and logical This, however, implies that the organism so operating must be capable of carrying out conversions of organic compounds Theoretically it should even be able to grow heterotrophically on the proper organic substrates Many of the photo-autotrophic organisms have yielded to this treatment, also a number of genuine or presumed chemo-auto-trophic organisms, such as hydrogen bacteria, certain sulfur bacteria, and iron bacteria, can develop satisfactorily in organic media in the absence of the specific oxidizable inorganic substance But there remain, as apparently insuperable obstacles, those cases which seem to fit Winogradsky's most rigorous definition of the chemo-autotrophs *par excellence,* i e , organisms which, at best, cannot utilize organic compounds, and are often harmed by them Clearly, if auto-trophs exist which cannot metabolize any organic material, this fact alone would suffice to disprove the second possibility

However strong and convincing the arguments in favor of the occurrence of such organisms may have seemed, such claims can no longer be considered con-clusive On the contrary, a number of recent studies have shown that the ex-perimental evidence points decisively in the opposite direction First, Cataldi has successfully cultured *Beggiatoa alba*—for which Keil (16) had maintained the ineffectiveness of organic substances, and the utilization of carbon dioxide as only carbon source—in sulfide-free organic media (63) Secondly, also the iron bacteria of the genus *Leptothrix* are, in pure culture, not dependent upon the presence of ferrous ions but can grow at the expense of organic matter (64) Finally, even the repeatedly substantiated reports that the nitrifying bacteria, as well as certain *Thiobacillus* species, cannot metabolize organic substances have been refuted Boltjes (17) had observed a favorable influence of "Nährstoff Heyden," an egg-albumen product, and some of the fatty acids on the growth of nitrifying bacteria Later, Bömeke (65) showed that, in the absence of am-monia and nitrite, these organisms actually carry out an oxidation of organic substances The occurrence of a similar process in *Thiobac thiooxidans* has also been proved (66, 67) It must, consequently, be conceded that even these most typical of chemo-autotrophic bacteria have failed to furnish experimental facts which would rule out the hypothesis that the synthesis of cellular materials proceeds by way of secondary conversions of one or a few primary assimilation products It is true that the above experiments with nitrifying bacteria and *Thiobac thiooxidans* have only shown that previously synthesized organic matter can be oxidized by these bacteria themselves Also, the rate at which such oxidations occur is very low Attempts at increasing this rate by the addition of various organic substances which might function as metabolites have, so far, not been promising Nevertheless, it seems well within the realm of possibility that future work in this direction may establish the existence of specific types of

organic compounds or of the need of particular environmental conditions to accomplish this end   Then it might ultimately become possible to provide the organisms with an organic medium, devoid of oxidizable inorganic energy sources, in which development can be secured   It should, however, be emphasized at this point that a successful achievement of this program must not be interpreted to mean that, under natural conditions, one could find the organisms growing heterotrophically wherever the proper organic materials and conditions are present, ecological considerations show that in nature their development and distribution would be restricted to those environments where the specific inorganic substrate occurs.

The ability of the nitrifying bacteria to oxidize organic materials has suggested to Bömeke that this function might explain the wide discrepancies in previous determinations of the ratio ammonia (nitrite) oxidized to carbon dioxide assimilated   For if a culture were analyzed some time after the oxidation of the inorganic substrate had been completed a considerable fraction of synthesized organic matter might meanwhile have been re-oxidized   It cannot now be decided for which experiments this would hold true, but it is certain that future work on this phase must pay close attention to this possibility

At first sight, Ruhland's data on the hydrogen bacteria appear to contradict this idea because the ratio hydrogen oxidized to carbon dioxide reduced is definitely not a function of time   However, the unusual experimental conditions provide a satisfactory answer to this situation   In all cases the amount of hydrogen in the cultures was considerably in excess of the available oxygen, so that the bacteria were never exposed to conditions where oxidation of cell materials could supersede the chemo-autotrophic reactions for lack of oxidizable inorganic substrate.   This may partly account for the high efficiency values observed with the hydrogen bacteria

In addition to the just discussed uncertainty attaching to the evaluation of the previously determined substrate—carbon dioxide ratios, another factor must also be considered   It has already been remarked that in long time experiments the oxidation of an inorganic substrate will be accompanied by a further conversion of a primary assimilation product which may entail appreciable losses   In that event all previously established values for such ratios are low   In view of what has been said earlier in connection with the metabolism of the hydrogen bacteria (p 344–345) it is clear that even the simultaneous determination of substrate, oxidation product, oxygen consumption, and carbon dioxide assimilation could not overcome this difficulty   At present the closest approximations could be achieved by short-time experiments in which due consideration is given to experimentally determined corrections for the concomitant oxidation of organic substances

5 *The evolutionary significance of chemo-autotrophic organisms*   The discovery of chemo-autotrophic bacteria showed that living organisms can exist and grow in a mineral environment and in the absence of light   The assumption that such a combination of factors occurred on earth when the surface had cooled down far enough to permit the existence of living beings has led to specu

lations in which the chemo-autotrophic bacteria were considered as the most "primitive" of all known organisms   The most interesting reconstruction of an evolutionary scale among microbes is unquestionably Orla Jensen's attempt (68), based upon the consistent use of such biochemical premises   In this and similar schemes it is taken for granted that, at the time when living organisms could start inhabiting the earth, organic matter must of necessity have been lacking   Recently, however, Oparin has exposed the fallacy of this premise (69), and pointed out that the synthetic mechanisms of the known autotrophic organisms in general appear so much more complex than those of heterotrophs that, on biochemical grounds, one could make out a good case for the thesis that the most primitive of bacteria must have belonged to the latter category   The simple nutrient requirements of the chemo-autotrophs certainly do not justify the conclusion that hence their metabolism is simple!

Metabolism is governed by enzyme systems, and many of the known enzymes contain active groups which have been identified with vitamins and growth factors (51, 70)   It follows that a knowledge of the vitamins required or synthesized by an organism may make it possible to draw inferences as to its metabolic activities   Although only a single investigation on the synthesis of vitamins by strictly chemo-autotrophic bacteria has been published to date, the results are of great interest in that they substantiate the supposition that there exists a close similarity in the metabolism of autotrophic and heterotrophic organisms   O'Kane (71) has established that *Thiobac thiooxidans*, grown autotrophically in a sulfur-containing medium, contains thiamin, riboflavin, nicotinic and pantothenic acids, pyridoxin, and biotin, i e , practically the full complement of B-vitamins whose function in the metabolism of organic compounds has either been elucidated or can be readily surmised   This fact militates strongly against considering the metabolism of these bacteria as "primitive "   The ability of all known hydrogen bacteria and many other potentially autotrophic organisms to develop with a variety of organic compounds makes it very likely that in these species the conditions would not be materially different   The peculiarities in the metabolism of the nitrifying bacteria make the latter a tempting group of organisms for an extension of this type of investigation

If, therefore, as the present trends indicate, organic compounds were present before life appeared on the earth, one can readily subscribe to Oparin's further deductions to the effect that purely chemical interactions between such substances may have resulted in the formation of a number of biocatalysts before a wholesale destruction of organic matter by living microbes became possible, and that, consequently, the earlier conditions may have been more conducive to the development of heterotrophic than of autotrophic bacteria

On the other hand, it must be realized that the foregoing statements do not necessarily imply that the metabolism of the heterotrophic micro-organisms is the more primitive   It becomes increasingly evident that the fundamental biochemical activities which the now living organisms display are found universally distributed   This makes it impossible to infer the course of evolution from such general biochemical considerations

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INTERRELATIONS BETWEEN THYROID FUNCTION AND VITAMIN METABOLISM

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During the past fifteen years considerable literature has accumulated on the interrelationships between the function of the thyroid gland and vitamin metabolism. Most of these studies are experimental, although some clinical observations have been reported. The experimental observations can be divided into two main groups: 1, the effect of a specific vitamin deficiency on the thyroid gland; 2, the effect of administration of a vitamin on animals which were given desiccated thyroid, or injected with thyroxin, or with the thyrotropic hormone of the anterior pituitary gland.

Hyperthyroidism, both experimental and clinical, has been shown to increase the requirements for certain vitamins. This increased vitamin requirement should not imply an antithyrogenic effect, although the increased requirements for certain vitamins indicate that some of the symptoms of hyperthyroidism, previously seen in animals and man, may be due to a secondary deficiency of these vitamins. The administration of these vitamins in larger amounts will correct these secondary changes, as will be pointed out in certain cases. An actual antithyrogenic effect has only been definitely shown in a few cases, although other suggestive evidence is available, which awaits confirmation and elucidation.

THYROID GLAND AND VITAMIN A. *1 Effect of deficiency of vitamin A on the thyroid gland* McCarrison (1931) found hypertrophy of the rat thyroid to occur during vitamin A deficiency. The thyroid gland of rats and guinea pigs has also been reported to be enlarged and inactive as a result of vitamin A deficiency (Mitzkewitsch, 1934, Uotila 1938, Schulze and Hundhausen, 1939a) However, other authors were unable to find thyroid hypertrophy during vitamin A deficiency (Drennan, Malcolm and Cox, 1931, Sampson and Korenchevsky, 1932, Sure, 1938) Sampson and Korenchevsky did, however, observe hypertrophy of the thyroid gland when the diet was deficient in both vitamin A and iodine.

Coplan and Sampson (1935) have studied this problem in the greatest detail and with adequate control observations. Their studies show the important influence of sex, time interval and diet in studying such problems. They found that iodine deficiency alone produced thyroid hypertrophy in male and female rats, which persists longer in the female, and is followed by atrophy in both sexes. Vitamin A deficiency produced a definite hypertrophy in female, but consistent atrophy in male rats. Whereas iodine deficiency and vitamin A deficiency produced hyperplasia of the epithelial cells of the thyroid gland, vitamin A deficiency also produced degeneration of the epithelial cells, which the authors

believe is a specific effect of vitamin A deficiency   A deficiency of both iodine
and vitamin A produced hypertrophy in male and female rats followed by
atrophy in the male   Harris and Remington (1939) observed that neither vita-
min A nor carotene influenced the utilization of minimal amounts of iodine in
the rat

2 *Effect of vitamin A administration on the thyroid gland*   As early as 1923
McCarrison found that the administration of cod liver oil would delay the meta-
morphosis of tadpoles receiving a high iodine intake, and later carotene was re-
ported to exert a similar effect in delaying the metamorphosis of axolotls (Rokhl-
ina, 1936)   It was also found that vitamin A would depress the accelerating
effect of thyroxin on the metamorphosis of tadpoles (Eufinger and Gottleib, 1933,
1935) and of salamander larvae (Fleischmann and Kann, 1936)   These results
demonstrate an antagonistic relationship between vitamin A and the thyroid
gland in amphibia

Harvey (1927) found that feeding cod liver oil, which contained some iodine,
to goats increased the amount of iodine in the milk more than an equivalent
amount of iodine as potassium iodide   The administration of large amounts of
vitamin A was reported by Sherwood, Toth and Carr (1934) to reduce the
amount of colloid in the thyroid gland of rats, a similar but not as marked a result
being obtained with caritol and haliver oil (Sherwood and Luckner, 1935)
Uotila.(1938) observed that an excess of vitamin A produced a diffuse hyperplasia
of the thyroid   Collazo and Rodriguez (1933) also reported exophthalmos in
rats receiving large doses of vitamin A   Schulze and Hundhausen (1939a) ob-
served a decreased thyroid activity and decreased thyrotropic content of the
anterior pituitary during hypervitaminosis A   According to Wegelin (1939)
and Elmer, Giedosz and Scheps (1935) vitamin A does not materially alter the
thyroid gland, although in some cases smaller follicles were observed   The addi-
tion of 1 per cent cod liver oil to the stock diet of young rats for 4 months signif-
icantly reduced the thyroid weight, when compared with rats on the stock diet
alone (Freudenberger and Clausen, 1935)

Hypervitaminosis A can be produced in rats by administering 8,000 to 40,000
I U vitamin A per day (Drigalski, 1933, Drigalski and Laubmann, 1933, Moll
et al , 1933)   Fasold and Peters (1933) administered 1 cc of Vogan[2] per day to
rats and produced symptoms of hypervitaminosis A which they reported could
be prevented, or cured before paralysis occurred, by administering thyroxin
However, Baumann and Moore (1939) and Weslaw and Wroblewski (1939)
could not confirm these results in fact, they both found that the symptoms of
hypervitaminosis A were enhanced by injections of thyroxin

3 *Effect of vitamin A on weight loss of hyperthyroid animals*   In an extensive
series of studies Abelin (1930a, 1930b, Abelin, Goldener and Kobori, 1926,
Abelin, Knochel, and Spichtin, 1930) showed the beneficial effects of yeast, egg
yolk, cod liver oil, high protein and high fat diets on the weight of rats fed thy-
roid gland   These authors were interested in finding a beneficial diet for use

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[2] Vogan, used extensively in German literature, 1 cc contains about 6 gamma of iodine,
40,000 rat units of vitamin A, vitamin D potency not stated

during hyperthyroidism and not in the effect of any particular compound   Von Euler and Klussman (1932) found that rats injected with thyroxin lost weight less rapidly when carotene was administered each day   According to Loumos (1934) a daily dosage of 10,000 U S P units of vitamin A administered to rats receiving 100 mgm of thyroid gland per day had little effect on the loss of weight An initial loss of weight was prevented for a few days after which time the loss of weight continued at about the same rate as control rats   Others have observed that vitamin A, administered in various forms, will affect the loss of weight of hyperthyroid animals to various degrees, either only partially or nearly completely preventing the loss of weight (Abelin, 1935, Schneider and Widmann, 1935, David, 1938, Steffan and Zois, 1938)   Vitamin A in arachis oil, or arachis oil alone would prevent the toxic action of thyroxin (Fasold and Peters, 1933)   These results also depend to a large degree on the dosage of thyroxin or thyroid gland employed, and the age and sex of the rats used   Rats weighing over 140 grams will rapidly lose weight when 100 mgm of thyroid gland are fed per day, whereas rats averaging 100 grams will gain weight for a while   Also, 100 mgm of thyroid gland per day will produce a more rapid loss of weight than will an injection of 100 gamma of thyroxin each day, and will thus offer a more rigorous method of testing the effect of vitamin A on weight loss. The sex differences in hyperthyroid rats can be seen from the data presented by Drill (1938b) and Logaras and Drummond (1938)   The latter authors administered 19 doses of 100 gamma of thyroxin to rats over a period of 28 days.   With thyroxin, and without vitamin A in the diet their male rats lost an average of 11 0 grams in weight, and with 3000 I U of vitamin A per day the hyperthyroid male rats lost an average of 8 6 grams   In the meantime normal male rats receiving 3000 I U vitamin A per day gained an average of 21 0 grams   On the other hand, female rats injected with thyroxin without vitamin A in the diet lost only an average of 1 6 grams whereas hyperthyroid female rats receiving 3000 I U vitamin A per day gained an average of 2 6 grams   Normal females receiving the same amount of vitamin A gained an average of 7 0 grams   Their other experiment with rats receiving 10 I U vitamin A per day showed a similar result   Although vitamin A may have some protective effect, under specific conditions, against the loss of weight of hyperthyroid animals, it is not as great as generally interpreted in the literature   The slight effect of vitamin A on weight reported by Logaras and Drummond was obtained by the use of small doses of thyroxin   Sure and Buchanan (1937b) showed an increased requirement for vitamin A during hyperthyroidism as judged by the appearance of xerophthalmia, but did not obtain any effect of moderate doses of vitamin A on the loss of weight of the rats

According to Weslaw and Wroblewski (1939) rats injected with thyroxin survived longer if vitamin A (as Vogan) was administered. However, they reported an equally good effect by administering sesame oil, the solvent for the vitamin A in Vogan   Thus the effect seems non-specific due to the administration of fat   In fact, the authors state the rats receiving sesame oil were in better condition, had better growth curves and survived longer than those fed the vitamin A supplement

Wegelin (1939) found that whereas thyroxin increased the number of mitotic figures in the liver, this increase could be prevented by vitamin A   An excess of vitamin A is also reported to reduce the protective action of thyroxin against the toxicity of acetonitrile in the Reid-Hunt reaction in mice (Fleischmann and Kann, 1936)   Greaves and Schmidt (1936) obtained evidence of an increased utilization of vitamin A in the hyperthyroid rat   In the thyroidectomized rat the requirement for vitamin A was decreased

4 Thyroid activity and storage of vitamin A   Euler and Klussman (1932) obtained a reduction of vitamin A and carotene in the liver when thyroxin was administered   When guinea pigs were fed a vitamin A free diet, the administration of carotene led to the storage of vitamin A and carotene in the liver, but when both carotene and thyroxin were given the liver failed to show storage of vitamin A and carotene (Abelin, 1933, Fasold and Peters, 1933)   Although Schneider and Widmann (1935) found no carotene and little vitamin A in the liver of hyperthyroid rats, they obtained an increase in liver carotene in thyroidectomized guinea pigs injected with thyroxin   If this report were confirmed it might mean that in the absence of the thyroid gland, even though thyroxin was injected, carotene could not be metabolized to vitamin A   According to Schneider and Widmann (1934) the injection of thyrotropic hormone will also lower the vitamin A stores in the liver of rats and guinea pigs   Logaras and Drummond (1938) found no reduction of hepatic vitamin A in rats injected with thyroxin, although the amount of thyroxin used was small   The administration of thyroxin to scorbutic animals did not cause a reduction of hepatic vitamin A (Balassa and Szanto, 1938)   Also, feeding large doses of vitamin A, Baumann and Moore (1939) did not observe any reduction of vitamin A in the liver when thyroxin was injected

In human cases of exophthalmic goiter normal values or an increase in hepatic stores of vitamin A have been reported   Wolff (1932) observed an increase in hepatic vitamin A in this disease, the mean value for 5 cases of exophthalmic goiter being 210 I U per gram, whereas in accidental death the value was 147 I U per gram   A similar result was obtained by Moore (1937), four exophthalmic patients showing an average of 304 I U per gram of liver, the values after accidental death being 210 I U per gram

5 Effect of vitamin A on carbohydrate and protein metabolism in hyperthyroid animals   Abelin, Knochel and Spichtin (1930) reported that vitamins A and D would prevent the fall of muscle and liver glycogen of rats generally produced by thyroid feeding, and a similar result was obtained with carotene (Abelin, 1935)   David (1938) also reported that vitamin A tended to reduce the carbohydrate changes of hyperthyroid animals to a minimum   Carotene was able to counteract the decrease of liver glycogen produced by thyrotropic hormone in guinea pigs but vitamin A did not prevent the disappearance of liver glycogen when thyroxin was administered (Schneider, 1934, Schneider and Widmann, 1934)   Microscopically, Wegelin (1939) showed some effect of vitamin A on liver glycogen, whereas the liver glycogen decreased when both vitamin A and thyroxin were administered, it did not altogether disappear, as it did when thy-

roxin was administered alone. However Steffan and Zois (1938) and Fasold and Peters (1933) did not obtain any effect of vitamin A on the liver glycogen of hyperthyroid rats It was also reported that carotene did not prevent the fall of liver glycogen of thyroidectomised guinea pigs injected with thyroxin (Schneider and Widmann, 1935)

Vitamin A has been shown to lessen the creatinuria seen during hyperthyroidism (Fischer and Oehme, 1933, 1937, De la Bernardie, 1939), and also to prevent the decrease of creatinine in heart and skeletal muscle (David, 1938), but one group of workers were unable to find any effect of vitamin A or carotene on the creatinuria (Steffan and Zois, 1938)

Török and Neufeld (1938) noted that the effect of thyroxin in lowering the serum lipase content of rabbit serum could be prevented by the simultaneous administration of large doses of vitamin A, but the effect was only temporary, for the serum lipase began to fall in a few days, even though the administration of vitamin A was continued.

θ Relation of thyroid function to carotene metabolism This part of the review is not concerned with any antithyrogenic properties of carotene or vitamin A, but with the effect of thyroid gland function on the conversion of carotene to vitamin A. Noorden (1907) first suggested the association of carotenemia with metabolic disturbances Since then various authors (Hess and Meyers, 1919, Head and Johnson, 1921, Stoner, 1928) have noted the appearance of carotenemia in certain individuals, and were unable to reproduce this condition by over feeding of vegetables to control patients In the meantime Kunde (1926) had noted the appearance of xerophthalmia in rabbits, on a diet that was adequate for normal rabbits, after they had been thyroidectomized for 8 to 12 months Her result can only be interpreted now to show that in the absence of the thyroid gland a molecule of carotene was not converted into two molecules of vitamin A, with the resultant occurrence of xerophthalmia

In 1932 Fellenberger and Grueter reported that the milk of thyroidectomized goats remained yellow, which was attributed to the presence of a lipochrome, probably carotene A year later Fasold and Heidemann showed that thyroidectomy in goats decreased the vitamin A content of goat's milk and increased the carotene content, leading to the conclusion that the thyroid gland is necessary for the conversion of carotene to vitamin A In the face of this evidence and the work of Kunde it seems certain that in the absence of the thyroid gland carotene is not metabolized to vitamin A, although the work of Kunde should be repeated using synthetic diets with carotene as the only source of vitamin A

Wendt (1935a) also believes that the thyroid is necessary for the conversion of carotene to vitamin A and for the storage of vitamin A in the liver Anderson and Soley (1938) reported 13 patients with carotenemia, 5 of which were hypo thyroid, 1 hypothyroid case showed hepatic insufficiency, and three other cases showed abnormal liver function Savy et al (1938) observed 5 cases of carotenemia with liver damage, and later (1939) one case of carotenemia with hypothyroidism. By studying the dark adaption of hypothyroid patients Wohl and Feldman (1939) also obtained evidence that the conversion of carotene to vitamin

A was disturbed   Escamilla (1942) reported 7 cases and Mandelbaum et al (1942) reported 1 case of hypothyroidism associated with carotenemia   As thyroid medication was instituted the blood carotene level fell

Since carotene is a lipochrome its metabolism might be expected to follow the metabolism of other lipochromes during disease   Although carotenemia was observed in hypothyroid patients, some functioning thyroid tissue is still left in these patients   This then raises the question as to cause of the hepatic damage seen in some of these patients   Is it due to a long standing thyroid hypofunction or the effect of an excess of carotene on the liver?   Since carotenemia was also seen in patients without evidence of hypothyroidism, but with hepatic damage, it is possible than an abnormal liver function alone may be the cause of the carotenemia   These problems still await experimental elucidation

7 *Effect of vitamin A on metabolic rate during experimental hyperthyroidism* There is a good deal of evidence to show that vitamin A partially counteracts the high oxygen consumption produced by thyroid feeding   Rappai and Rosenfeld (1935) increased oxygen consumption 42 4 per cent by injecting thyroxin, whereas a rise of only 13 per cent was obtained when both vitamin A and thyroxin were administered, and an increase of 10 per cent when carotene and thyroxin were given   Sesame oil had no effect on the B M R   Abelin (1935) obtained a similar increase of 50 per cent in oxygen consumption by administering thyroxin and only 20 per cent rise when vitamin A and thyroxin were administered to rats   These results were confirmed on rats by Logaras and Drummond (1938) and Belasco and Murlin (1940)   In an acute study on cats, the injection of thyroxin for three days increased O_2 consumption 45 7 per cent, and with carotin and thyroxin the rise was only 25 4 per cent (*Smith and Perman, 1940*)

The R Q of hyperthyroid rats was not affected by vitamin A (Rappai and Rosenfeld, 1935), nor did carotene influence the R Q of cats injected with thyroxin for 3 days (Smith and Perman, 1940)   This suggests that vitamin A did not selectively affect the metabolism of any particular foodstuff   Belasco and Murlin (1940) also reported that the increase in liver and kidney metabolism seen in hyperthyroidism was not changed by vitamin A.

Chevalier and Baert (1934) reported that vitamin A deficiency increased the B M R of rats, and that large doses of vitamin A decreased the metabolic rate below normal   However, Sherwood, Toth and Carr (1934), Belasco and Murlin (1940) and Sheets and Struck (1942) did not find any affect of vitamin A on oxygen consumption of normal rats   The latter authors also found no significant change in thyroidectomized rats fed large doses of vitamin A   Rappai and Rosenfeld (1935) found that vitamin A lowered the B M R of normal rats 4 to 12 per cent

Sheets and Struck (1942) confirmed the above reports, finding that after premedication with vitamin A for 50 days thyroid feeding raised the B M R of rats an average of 25 5 per cent, whereas other rats receiving only thyroid showed a rise of 58 per cent   However, after the B M R of these rats had risen to 58 per cent treatment with vitamin A showed a tendency to lower the level, but did not produce any significant change   Thus, while the administration of thyroid

and vitamin A together will prevent the rise in oxygen consumption to the level produced by thyroid alone, there is no experimental evidence to show that treatment with vitamin A will reduce the high B M R., once it has been produced by thyroid feeding

8   Thyrotropic hormone of the anterior pituitary gland and vitamin A   There are reports which indicate that vitamin A will partially prevent the histological changes produced in the thyroid gland of guinea pigs by injection of thyrotropic hormone (Schneider, 1934, Fellinger and Hochstaedt, 1936, Elmer, Giedosz and Scheps, 1935)   Parhon and Werner (1935) have reported that thyroxin or thyrotropic hormone reduced the blood carotene in ducks   According to Schulze and Hundhausen (1939a) a lack of vitamin A leads to increased thyroid activity and increased thyrotropic potency of the pituitary gland, whereas hypervitaminosis A has the opposite effect

9   Clinical studies on thyroid function and carotene and vitamin A metabolism   Mellanby and Mellanby reported (1921b) that patients with exophthalmic goiter receiving a diet high in cod liver oil and low in fat gained weight   Although no figures were given, the B M R   was also reported to be lowered   Others have also studied the effect of iodized fatty acid and vitamins A and D in the treatment of Graves' disease (Adamson and Cameron, 1928, Fraser and Cameron, 1929, Rabinowitch, 1929)   Their results are complicated by the simultaneous administration of iodized fatty acids or of iodine contained in the cod liver oil

Wendt (1935a, 1935b) observed that patients with Graves' disease had practically no serum vitamin A and had low serum carotene values.   The administration of vitamin A to such patients caused less of a rise in plasma vitamin A than is seen in normal persons   These serum values were observed to increase after successful iodine treatment or surgery   He also found a low serum vitamin A value in cretins, which showed little change when carotene was administered orally   This confirms experimental work discussed above, where the thyroid gland was found to be necessary for the conversion of carotene to vitamin A. Clausen and McCoord (1938) observed two hyperthyroid patients with low plasma vitamin A and carotinoids   The majority of their hypothyroid patients showed a high plasma carotene value, and in all cases the vitamin A value was low   When several of the hypothyroid patients were studied, following thyroid therapy to elevate the B M R. and lower blood cholesterol, the carotene and vitamin A values tended to return to normal   Wendt (1935c) also reported that large doses of vitamin A (as Vogan[2]) increased the weight and lowered the B M R. from 46 per cent to 20 5 per cent in three hyperthyroid patients, and two other patients showed some improvement (cf Wendt, 1936)   Dietrich (1936) also found vitamin A to be beneficial in three of six hyperthyroid patients Abelin (1936) advocates the use of vitamin A in conjunction with iodine and diiodotyrosine as an aid in controlling crisis and the temporary exacerbation that may occur under such treatment

Studies on the dark adaptation of hyperthyroid patients also indicated a deficiency of vitamin A in hyperthyroid patients (Wohl and Feldman, 1939) Evidence was obtained which suggested that the administration of thyroid ex-

tract or a-dinitrophenol facilitates the utilization of vitamin A by the visual mechanism, representing an increase in the speed and extent of visual purple regeneration (Patek and Haig, 1941)   Godtfredson (1941) found impaired dark adaptation and liver function in ten hyperthyroid patients   He suggests that the liver damage in hyperthyroidism disturbs vitamin A metabolism.

Fasold (1937) treated 7 girls with adolescent goiter, none showing any symptoms of hypo- or hyperthyroidism, with Vogan, administering 30 drops 3 times a day   After about one month of treatment the tumor had regressed, but recurred a few weeks after treatment was discontinued   The dose of Vogan used supplied about 3 6 gamma of iodine per day

In 1938 Catel reported a case of hyperthyroidism in a child which was treated with vitamin A   The blood level of vitamin A was raised but the B M R and other symptoms were not affected   Jacobi and Pomp (1938) studied 14 cases of hyperthyroidism, some treated by rest and diet, and others treated by rest, diet and the administration of vitamin A as Vogan   The criteria of improvement they used were decreased B M R and pulse rate, increase in weight, and improvement of general condition, but they did not find any beneficial effects from the vitamin A supplement

Although experimental evidence shows that vitamin A has some relation to thyroid function, the clinical results are as yet too few to draw any conclusions concerning the efficacy of this treatment, and such factors as hospitalization, diet and rest   It should also be borne in mind that plasma carotene and vitamin A will vary from the norm in diseases other than thyroid disorders (Clausen and McCoord, 1938, Moore, 1937)

Summary   A deficiency of vitamin A will produce thyroid hypertrophy, although a sex difference in response is present   An excess of vitamin A seems to decrease the amount of colloid in the thyroid gland, although this result is still controversial, as is the effect of thyroxin on hypervitaminosis A   In amphibia, however, a definite antagonism between thyroxin and vitamin A has been demonstrated

Experimental hyperthyroidism in mammals increases the requirements for vitamin A   However, the degree to which the administration of vitamin A will prevent a loss of weight in such animals is not clear, especially as some authors have observed no effect of vitamin A on weight loss and others have obtained the same effect with the vehicle in which the vitamin A is dissolved   The effect of hyperthyroidism on the liver stores of vitamin A, and the effect of vitamin A on liver glycogen during hyperthyroidism still await further clarification, although vitamin A seems to decrease the creatinuria observed in such animals

All of the evidence to date indicates that in the absence of the thyroid gland carotene is not metabolized to vitamin A   Various authors also agree that the simultaneous administration of vitamin A with thyroxin will partially prevent a rise in the B M R , this being the first definite antithyrogenic action of vitamin A to be demonstrated   However, there is no evidence to indicate that the treatment of hyperthyroid animals with vitamin A will lower the B M R   There is some evidence to indicate that vitamin A will partially prevent the histological changes in the thyroid produced by the thyrotropic hormone

Clinically, hyperthyroidism increases the requirement for vitamin A, also lowering serum vitamin A and serum carotene   Although the administration of vitamin A to such patients will provide for the increased requirements, any other effect of vitamin A in such patients awaits further demonstration

THYROID GLAND AND VITAMIN B₁   *Effect of deficiency of vitamin B₁ on the thyroid gland*   McCarrison (1914) first reported hyperplasia of the thyroid gland of pigeons fed a polished rice diet. Later Spence (1932, 1934) observed thyroid enlargement in rats, the diet however containing multiple deficiencies There is also said to be a decreased amount of thyroid hormone in the thyroid gland during vitamin B deficiency (Verzar and Vasarhely, 1924) and Aalsmeer (1932) suggests that beriberi is associated with dysfunction of the thyroid gland According to Fischer (1933) a deficiency of both iodine and vitamin B₁ will produce thyroid hypertrophy   However, iodine deficiency alone will produce thyroid hypertrophy and will reduce the amount of iodine in the thyroid gland

A recent study by Sandburg and Holly (1933) revealed some interesting results   They fed rabbits an autoclaved diet of alfalfa hay and rolled oats and found that the thyroid increased in size and was very vascular in appearance They then made the observation that the administration of either iodine or yeast had an effect on the Ca and P metabolism of rabbits with hyperplastic thyroids. Both the iodine and yeast caused a marked increase in Ca excretion, changing the ratio of Ca and P retention from Ca P = 1 0.3 to Ca P = 1 216 Presumably the effect of the yeast on the Ca and P metabolism of rabbits with hyperplastic thyroid glands was due to its vitamin B₁ content, as no effect was obtained with autoclaved yeast

Other experiments on vitamin B deficiency have generally shown an increase in colloid, later, as the time of deficiency increased, followed by atrophy of the thyroid gland (cf Meyer, 1937, for earlier references,—Schneider, 1938a, Hund hausen and Schultze, 1939)   Sure (1938) reported an increase in thyroid weight in B₁ deficiency   No change in the size iodine content, dry matter, or structure of the thyroid gland of rats on a vitamin B deficient diet has been observed by Carpenter and Sharpless (1937) and Harris and Remington (1939)   Carpen ter and Sharpless also concluded that the colloid goiter developed in rats by feeding a low iodine and a vitamin B₁ deficient diet could be prevented by feeding more iodine or vitamin B   Harris and Remington failed to confirm this observa tion   They found no difference between the effect of yeast or autoclaved yeast on the thyroids of rats fed a low iodine diet, and supplements of vitamin B₁ failed to prevent the picture of colloid goiter from appearing   A diminished food in take per se is reported to have no effect on the thyroid gland (Hundhausen, 1939)

Spence (1934) found no effect of a vitamin B extract on size or vascularity of the thyroid gland of rabbits fed fresh cabbage and rolled oats

Baglioni (1940) reported that the administration of vitamin B₁ did not alter the histological appearance of the guinea pig thyroid, whereas Giedosz (1938a) has reported injections of vitamin B₁ in the guinea pig stimulate the thyroid gland

2 Effect of deficiency of other B vitamins on the thyroid gland   Giedosz (1938b) observed stimulation of the thyroid in guinea pigs during a deficiency of vitamin

B₂, and Sure (1938) reported that 88 per cent of the rats showed an average increase of 42 8 per cent in thyroid weight during a vitamin B₂ deficiency   Sure also found a slight increase in thyroid weight during a deficiency of vitamin B₆, and also found that 65 per cent of the rats showed a 28 per cent increase in thyroid weight on a B complex free diet   Uotila (1938) reported that a deficiency of the vitamin B complex produced thyroid atrophy, the follicles being enlarged and filled with colloid

3   Relation of B vitamins to calorie intake and weight during experimental hyperthyroidism   That a relationship exists between calorie intake and vitamin B₁ requirements has been demonstrated by many authors (cf Cowgill, 1934) Himwich, Goldfarb and Cowgill (1932) first studied the relation of calorie intake to the B vitamins during hyperthyroidism   They found that normal dogs fed a yeast-free diet developed anorexia in an average of 32 days, whereas hyperthyroid dogs fed a yeast-free diet developed anorexia in an average of 17 days These dogs started to regain their lost weight when 10 grams of a vitamin B concentrate was fed each day   Cowgill and Palmieri (1933) also found that the vitamin B requirement (as yeast) of pigeons was increased by feeding thyroid gland   This report has been amply confirmed (Capraro, 1937, Mouriquand, Morin and Czerszchowska, 1939, Morin and Czerszchowska, 1939)

It was then observed by Sure and Smith (1934) that a potent vitamin B concentrate protected rats receiving thyroxin from a loss of weight   With the advent of crystalline vitamin B₁ Sure and Buchanan (1937a) restudied the effectiveness of vitamin B₁ in *preventing* the loss of weight produced by hyperthyroidism Their tables show that 7 rats fed diet 3080, containing 15 per cent autoclaved yeast, and receiving 0 2 mgm of thyroxin (orally or parenterally?) per day gained 64 to 108 per cent of the weight of control rats when a supplement of 30 to 300 gamma of vitamin B₁ was administered each day   Rats on the same diet fed 0 5 gram of yeast per day plus 0 2 mgm of thyroxin per day gained 52 to 132 per cent of the weight of control rats   The change in weight of untreated rats receiving the same diet and thyroxin is not stated

Drill (1938b) studied the effect of vitamin B₁ and yeast on hyperthyroid rats that had lost weight, to determine if such treatment would enable them to regain their lost weight   When the rats had lost an average of 23 grams in weight they were injected with 500 gamma of vitamin B₁ per day   The vitamin B₁ stopped a further loss of weight, but the rats did not regain their lost weight until a rich source of the vitamin B₂ complex was administered   A sex difference in response was also observed   These results were confirmed and extended by Drill and Sherwood (1938)   They reported that the effect of vitamin B₁ in stopping further loss of weight in hyperthyroid rats was due to the increased calorie intake which it produced   Although vitamin B₁ increased food intake and prevented a further loss of weight, the rats still did not regain their lost weight until a rich source of the vitamin B₂ complex was supplied   Thus in addition to vitamin B₁, some other factors in the B₂ complex are also required in larger amounts than normal   If the rats were limited to 13 grams of food per day the vitamin B₁ injections did not stop a further loss of weight   The effect of vitamin B₁ in pre-

venting a further loss of weight of hyperthyroid rats was confirmed by Peters and Rossiter (1939)   It was later found that pyridoxine (vitamin B_6) and calcium pantothenate can effectively replace the B_2 complex addition to the diet of hyperthyroid rats, and the rats regained and maintained their lost weight while still receiving thyroid gland (Drill and Overman, 1942)   Thus in addition to vitamin B_1, both pyridoxine and pantothenic acid are required in larger amounts during experimental hyperthyroidism

When a small amount of yeast was fed to hyperthyroid dogs an initial increase in food intake was observed   The removal of the yeast from the diet after 4 to 9 weeks of thyroid feeding produced a sudden and marked drop in appetite to values below normal within a few days (Drill, 1941)   The subcutaneous injection of 2 mgm of vitamin B_1 restored the appetite to its previous hyperthyroid level within 24 to 48 hours   This suggests that anorexia is not a primary effect of hyperthyroidism but is a secondary symptom due to a deficiency of vitamin B_1

Hyperthyroid dogs that lost weight, following the removal of the yeast from the diet, require increased amounts of vitamin B_1 and the vitamin B complex in order to regain their lost weight (Drill and Shaffer, 1942), confirming previous work on rats   By administering sufficient B vitamins from the start of thyroid feeding it was found possible to maintain the weight of 3 of 4 hyperthyroid dogs for as long as 112 days   The food intake of such dogs is above normal   A similar maintenance of body weight of thyroid fed rats receiving yeast concentrate was obtained over a period of 79 days (Drill, Overman and Leathem, 1943)

4 Effect of B vitamins on estrous cycle during hyperthyroidism   Earlier studies have shown that thyroid feeding soon produces a constant diestrus in rats. Hyperthyroid rats receiving vitamin B_1 and yeast supplements were observed to maintain normal estrous cycles (Drill and Sherwood, 1938)   In further studies it was shown that hyperthyroid rats receiving a normal but small amount of yeast each day, had only two or three estrous cycles before entering complete diestrus   Besides a loss of weight, these rats also showed a fall in pituitary and ovarian weight, ovarian sections suggesting a lack of anterior pituitary hormone. Other rats received thyroid gland and yeast concentrate for 79 days with complete maintenance of weight and estrous cycles   The weights of the pituitary and ovaries were normal, as was ovarian histology (Drill, Overman and Leathem 1943)

5 The B vitamins and liver function during experimental hyperthyroidism   Youmans and Warfield (1926), using the phenoltetrachlorphthalein test, did not detect hepatic damage in hyperthyroid dogs.   With the use of more sensitive tests thyroid feeding was shown to produce an increase in bromsulphalein retention (Drill and Hays, 1940) and a rise in serum phosphatase (Drill and Shaffer, 1943)   Drill and Hays (1940) fed thyroid gland to 5 dogs receiving a small but normal amount of yeast.   Three of the dogs developed abnormal liver functions during the period of thyroid feeding, the dye retention being further increased by removing the yeast from the diet   The other two hyperthyroid dogs still

had a normal liver function during this period, and removing the yeast from their diet produced an abnormal dye retention within 24 hours   Although the deficiency of the B vitamins seems to bear a causal relationship to the production of hepatic damage in thyroid fed dogs, it does not mean that it is the only or the most important factor   Later studies on this problem were reported (Drill and Hays, 1942), showing that once an abnormal liver function had been produced in hyperthyroid dogs, treatment with large doses of vitamin B_1 and yeast concentrate did not improve the abnormal liver function   It was also shown that a yeast-free diet renders dogs extremely susceptible to the effects of thyroid feeding, an abnormal liver function being produced in an average of 22 days (Drill, Shaffer and Overman, 1943)   Thyroid fed dogs receiving a small amount of yeast each day developed an abnormal liver function in an average of 45 days When hyperthyroid dogs were fed a high B vitamin diet it took an average of 90 days before an abnormal liver function was observed   Thus, the amount of B vitamins fed has a direct relationship to the time elapsed before an abnormal liver develops   A high B vitamin diet will delay but will not prevent the appearance of an abnormal liver function in hyperthyroid dogs

6   The B vitamins and pulse rate during experimental hyperthyroidism   The effects of diets deficient in vitamin B on the cardiovascular system of man and animals have been discussed by Weiss and Wilkins (1937) and Frazier and Ravdin (1938)   In the dog and rat a deficiency of vitamin B_1 produces bradycardia, whereas in human beriberi bradycardia is rarely observed and tachycardia is common   Parade (1938b) reported that thyroid feeding during a deficiency of vitamin B_1 will retard the development of bradycardia and subnormal temperature   In experiments on dogs thyroid feeding produced a marked tachycardia of 150 to 160 beats per minute (Drill and Hays, 1942)   The removal of the yeast from the diet of these dogs after 30 to 60 days caused a marked drop in pulse rate to values below 100 beats per minute, while still feeding thyroid gland   The injection of these dogs with vitamin B_1 raised the pulse rate to its previous hyperthyroid level within 24 to 48 hours   Thus, the action of an excess of thyroid gland in producing tachycardia in dogs depends in some manner on an adequate supply of vitamin B_1, possibly through an effect on food intake (Parade, 1937, 1938a)

Hyperthyroid dogs fed a yeast-free diet develop only a slight tachycardia which returns to normal within 10 days (Drill, Shaffer and Overman, 1943), and hyperthyroid dogs receiving a high B vitamin diet maintain a high pulse rate for only 80 to 100 days, after which time the pulse rate falls towards normal   The drop in pulse rate at this time seems to be associated with a compensatory hypertrophy of the heart

7   The B vitamins and liver glycogen during hyperthyroidism   Abelin and coworkers first studied the effect of foodstuffs on liver glycogen during experimental hyperthyroidism (Abelin, 1930, Abelin, Knochel and Spichtin, 1930)   Among other findings, discussed elsewhere in this paper, they found that yeast would prevent the fall in liver glycogen of rats fed thyroid gland for 5 to 6 days   This observation was confirmed by Drill (1937) who studied the liver glycogen of rats

injected with small amounts of thyroxin   Frazier, Brown, and Vars (1939) obtained a partial rise of liver glycogen in one of three groups of hyperthyroid rats treated with yeast and McIver and Winter (1941) obtained maintenance of liver glycogen in 4 of 6 hyperthyroid rats treated with yeast   Drill, Overman and Shaffer (1942) also found that yeast concentrate would maintain the weight and liver glycogen of rats fed thyroid gland for 86 days   Vitamin B_1 is also reported to diminish the creatinuria produced in rats by injecting thyroxin (De la Bernardia, 1939)

8 Effect of hyperthyroidism on vitamin B_1 content of tissues   Rats fed 100 mgm of thyroid gland per day and 12 grams of a normal diet per day supplying 10.8 I U of vitamin B_1 per day showed a normal amount of vitamin B_1 in the spleen, a reduction in the kidney, and a marked reduction in the liver   The ability of hyperthyroid rats to store vitamin B_1 was studied in a similar group of rats injected with 500 gamma of vitamin B_1 during the last nine days of the experiment   When compared with control rats injected with vitamin B_1 the hyperthyroid rats showed a normal amount of vitamin B_1 in the spleen and muscle, a slightly raised content in the heart and a definite reduction in the kidney and liver   The storage in the whole liver was 35 7 per cent less than the controls (Drill, 1938a)   Peters and Rossiter (1939) confirmed the above report using rats injected with thyroxin and the castorulin test for vitamin B_1, and found a lowered amount of cocarboxylase in rat tissues, the greatest lowering again being observed in the liver   Hyperthyroidism in the rat does not increase the rate of absorption of vitamin B_1 from the intestine (Stockholm, Althausen and Borson, 1941)

Schneider and Burger (1938) found that control patients excreted 80 to 100 gamma of vitamin B_1 in the urine per day, being between 7 and 8 gamma per cent, with a serum value of 6 4 gamma per cent.   Hyperthyroid patients excreted an average of 8 4 gamma per cent of vitamin B_1 in the urine but with a daily output averaging 224.8 gamma per day   Coincident with this large increase in B_1 excretion was a daily urine output averaging 2770 cc   After operation the urine output sank to an average of 1100 cc with a normal value of 79 9 gamma of vitamin B_1 per day

9 Vitamins of the B_2 complex and the thyroid gland   The effect of a deficiency of the vitamins of the B_2 complex on the thyroid gland has been discussed above   In 1926 Kunde reported that thyroidectomized rabbits, receiving the same diet as normal animals, developed pellagra like lesions on the dorsum of the feet, the neck, mouth and nose, after 8 to 20 months of complete thyroidectomy   It is interesting that Greene (1938) observed two patients with myxedema and pellagra that showed a similarity of gastroenteric, neurologic, and psychic symptoms and to a certain extent, similar dermatologic manifestations   Drill and Overman (1942), studying the weight changes of hyperthyroid rats, did not find any evidence of an increased requirement of riboflavin, above that supplied by the daily yeast supplement   The increased requirements of pyridoxine and pantothenic acid during hyperthyroidism have been reported above

10 Thyrotropic hormone of the anterior pituitary gland and the B vitamins

Elmer, Giedosz and Scheps (1937) did not find any antagonistic effect of vitamin B_1 on the thyroid stimulation produced in guinea pigs with the thyrotropic hormone   Similar results were obtained by Schneider (1936b)   Cutting and Robson (1939) reported no effect of daily administration (length of administration?) of 40 I U of vitamin B_1 and 12 Sherman units of vitamin B_2 on the B M R of two guinea pigs injected with thyrotropic hormone   Vitamin B_1 did not prevent the death of guinea pigs or lessen the reduction of liver glycogen of guinea pigs injected with large doses of thyrotropic hormone (Schneider, 1938b)   Gentzen and Moire (1938) reported that premedication with vitamin B_1 lessens the effect of thyrotropic hormone on the B M R of guinea pigs

The thyroid gland of rats fed a B_1 deficient diet is said to respond normally to an injection of thyrotropic hormone (Schneider, 1938a), although extracts of the pituitaries of such rats, when tested on immature guinea pigs, were found to be less active than extracts from normal rats (Hundhausen and Schulze, 1939)

Elmer, Giedosz and Scheps (1938), Hoen and Oehme (1938), and Wahl (1939), showed that the administration of riboflavin (vitamin B_2) did not affect the B M R or histological picture of the thyroid of normal guinea pigs, or of guinea pigs injected with thyrotropic hormone   Wahl (1939) reported that riboflavin augmented the increase in B M R produced by thyrotropic hormone   Riboflavin deficiency does not affect the thyrotropic content of the pituitary (Schulze and Hundhausen, 1939)

11   Clinical studies on the B vitamins and thyroid activity   Shimazono (1931) reported that iodine and thyroid substance have a curative effect on the paralysis produced by vitamin B deficiency, but that these substances were not tolerated by patients with beriberi until the cardiac symptoms were first treated and relieved   In studying blood iodine, Takasugi (1931) found that in beriberi there was first an increase in blood iodine and B M R both of which later dropped

Means (1937) mentioned 3 hyperthyroid patients, studied by Lerman and Hertz, that gained weight when a high B vitamin diet was administered   In 1938 Frasier and Ravdin studied the effect of vitamin B_1 and yeast on the preoperative preparation of patients with Graves' disease   They found not only improved nutritional status, as judged by increased appetite and gain in weight, but also that "patients with severe hyperthyroidism can be adequately prepared for operation in a shorter time"   The patients treated with the B vitamins also showed a larger preoperative fall in pulse rate than control patients   No influence of the supplement of B vitamins was found on the B M R or on the severity of the post-operative reaction   Frazier and Ravdin administered about $2\frac{1}{2}$ times the estimated daily requirement of vitamin B_1 Experimental work indicates that the requirement for vitamin B_1 during hyperthyroidism may be larger than this (Drill and Shaffer, 1942), suggesting that larger doses of the B vitamins might be more beneficial   Scrutinio (1939) also reported that administration of 1000 I U of vitamin B_1 increased the weight and decreased the B M R of hyperthyroid patients   The author favors the use of vitamin B_1 in cases of hyperthyroidism with cardiac decompensation   Hertzler (1941) also uses vitamin B_1 in preoperative preparation of toxic patients, cardiotoxic

patients, and in thyroid crisis Jacobi and Pomp (1938) reported on 14 cases of hyperthyroidism, some were treated by rest and diet, while others received the same regimen plus the administration of vitamin A, either alone or in combination with vitamin B_1 They reported no apparent beneficial effects from the administration of vitamin A, either alone or in combination with vitamin B_1 The increased urinary excretion of vitamin B_1 in patients with Graves' disease has been discussed in section 8 of this review

Summary As various results have been reported no definite conclusion can be stated at this time, as to the effect of a vitamin B_1 deficiency of the thyroid gland However, the experiments of Carpenter and Sharpless, and Harris and Remington show no effect of vitamin B_1 deficiency on the thyroid gland, and in other cases the possibility of an iodine deficiency must be ruled out Hyperthyroidism increases the requirements for vitamin B_1, vitamin B_6 and pantothenic acid The administration of sufficient yeast concentrate to thyroid fed animals will prevent a loss of weight, or will allow hyperthyroid animals which have lost weight to regain their lost weight The maintenance of body weight is due to the increase in food intake that occurs when the increased requirements for the B vitamins are met Such animals will also maintain normal estrous cycles

Experimental hyperthyroidism has been shown to produce hepatic damage, which is related in part to the amount of B vitamins supplied in the diet Vitamin B_1 also shows a relation to the cardiovascular system in experimental hyperthyroidism, and yeast concentrate tends to maintain the liver glycogen of thyroid fed animals

Hyperthyroidism decreases the amount of vitamin B_1 in various rat tissues, and clinically there is an increased excretion of vitamin B_1 Vitamin B_1 does not appear to antagonize the administration of the thyrotropic hormone Clinically most authors have reported a beneficial effect of vitamin B_1 and yeast in patients with Graves' disease, although one paper reported no effect when vitamin B_1 was administered

THYROID GLAND AND VITAMIN C *1 Effect of a deficiency of vitamin C on the thyroid gland* In 1915 Rondoni and Montagnani observed hemorrhagic infiltration of the thyroid gland of guinea pigs on a vitamin C deficient diet. McCarrison (1920a, b) confirmed this finding and also noted an increase in thyroid weight of scorbutic guinea pigs, sometimes 2 or 3 times normal An irregular enlargement of the thyroid was also observed by Bessesen (1923) However, Löwy (1923) did not find changes in the thyroid gland of guinea pigs during scurvy, and Meyer (1928) reported irregular changes in the thyroid of guinea pigs fed a vitamin C deficient diet for 30 days and concluded that no noteworthy changes took place.

Harris and Smith (1928) reported an increase in both the height of the follicular epithelium and number of inter follicular cells, with a decrease in amount and an increase in the vacuolation of the colloid, after *chronic* scurvy lasting 97 days They also suggested a possible influence of vitamin C on iodine metabolism Abercrombie (1935) confirmed the findings of Harris and Smith, also observing hemorrhagic infiltration to a slight extent in normal animals, more so

in *acute* scurvy of 29 days' duration, and much more marked in 126 days of *chronic* scurvy   He further found that potassium iodide or thyroid gland, administered during acute or chronic scurvy, exerted their characteristic effect on the thyroid gland, without prolonging the life of the vitamin C deficient guinea pigs, and concluded that vitamin C is not concerned in iodine metabolism   Neither Rondoni and Montagnani, Löwy, Harris and Smith, nor Abercrombie found any effect of starvation on the histology of the guinea pig thyroid

May (1937) also found that vitamin C deficiency in the guinea pig produced a marked hyperemia, somewhat increased activity, irregular structure, and the formation of utricles and vesicles without colloid   The therapeutic or prophylactic administration of vitamin C would prevent these changes   Other reports on vitamin C deficiency in the guinea pig have also shown hyperplasia and hypersecretion of the thyroid gland, which was more marked in chronic than acute cases (Schulze and Linnemann, 1938, Uotila, 1938)   Vitamin C did not prevent the hyperplasia produced by feeding fresh cabbage to rabbits (Spence, 1934)

2   Increased requirements of vitamin C and effect of vitamin C during experimental hyperthyroidism   Whereas the administration of 0 5 mgm of ascorbic acid per day would prevent the loss of weight of scorbutic guinea pigs, Demole and Ippen (1935) reported that 10 to 20 mgm per day were required when the animals were injected with 0 1 mgm of thyroxin per day   Fischback and Terbrüggen (1937b) found that thyroxin administration to scorbutic guinea pigs increased the loss of weight, but that if large doses of ascorbic acid were given before the thyroxin the loss of weight was less   Ray (1938) also reported that the requirements of thyroid-fed rabbits for vitamin C was increased   Vitamin C deficiency and hyperthyroidism are also said to produce the same blood picture in guinea pigs (Aszodi, 1937)   Brandt (1937) observed that the effect of thyroid gland on the axolotl was less when vitamin C was also given   It is also reported that vitamin C will reduce the blood iodine of normal and hyperthyroid animals (Lohr, 1936)

3   Effect of vitamin C on carbohydrate and protein changes in hyperthyroid animals   Fischbach and Terbrüggen (1937a) found that the effect of thyrotropin in lowering liver glycogen was inhibited by large doses of vitamin C, but that vitamin C did not prevent the fall in liver glycogen when thyroxin was administered   Large doses of ascorbic acid were also reported to prevent a loss of muscle glycogen and partially prevent a fall of liver glycogen produced by thyroxin (Steffan and Zois, 1938), and would prevent or suppress the loss of muscle glycogen produced in guinea pigs by injecting thyrotropic hormone

The creatinuria observed during hyperthyroidism has been found to be reduced by the administration of vitamin C (Fischer and Oehme, 1937, v Plehwe, 1938, Steffan and Zois, 1938, De la Bernardia, 1939)   Similar results have also been reported after the administration of thyrotropic hormone (Steffan and Zois, 1938)

Vitamin C will not prevent the fall in serum lipase induced in rabbits by thyroxin (Török and Neufeld, 1938)

4 Thyroid activity and tissue vitamin C Many authors have reported a decrease of vitamin C in the adrenal during hyperthyroidism (Demole and Ippen, 1935, Mosonyi, 1935, Plaut and Bülow, 1935, Nespor, 1936, Farmer and Fribourg, 1942) Farmer and Fribourg believe that the effect of thyroid feeding in increasing the anaphylactic response of guinea pigs is due to the reduction of adrenal vitamin C, which in turn lowers the cortin content of the adrenal This hypothesis, however, requires further testing as to the therapeutic administration of vitamin C in preventing the changes reported Contrary to all of the above reports Paal and Brecht (1937) reported that the administration of thyroxin and thyrotropic hormone to guinea pigs increased the amount of vitamin C in the adrenal, and Ray (1938) observed no decrease in vitamin C in adrenals of thyroid fed rabbits. Mosonyi (1936) found that the loss of vitamin C in the liver and adrenals of guinea pigs after thyroxin could be prevented by vitamin A In dogs, hyperthyroidism also reduced the vitamin C content of the liver and adrenals, whereas thyroidectomy produced the opposite results (Thaddea and Runne, 1938) Sure and Theis (1939) observed that the reduction of vitamin C in various rat tissues produced by thyroxin was accentuated when a supplement of vitamin B_1 was not administered

5 Effect of vitamin C on metabolic rate during experimental hyperthyroidism Demole and Ippen (1935) and Oehme (1936) found that vitamin C would partially reduce the high B M R. seen in hyperthyroidism This was confirmed by Belasco and Murlin (1940), who also reported that the increased metabolism of liver and kidney during hyperthyroidism was not influenced by vitamin C

6 Thyrotropic hormone of the anterior pituitary gland and vitamin C The administration of ascorbic acid is said to produce changes in the thyroid gland similar to that induced by thyrotropic hormone (Heyl, 1934) Schulze and Linnemann (1938) did not find an increase of thyrotropic hormone in the pituitary gland during vitamin C deficiency

There is some evidence that vitamin C will partially inhibit the action of the thyrotropic hormone in the thyroid gland (Marine, Baumann and Rosen, 1934, Elmer, Giedosz and Scheps, 1935, Schulze and Linnemann, 1938) Other authors, however, did not obtain any effect of large doses of vitamin C on the thyroid hyperplasia produced by thyrotropic hormone (Spence and Scowen, 1935, Schäffer, 1936, Schöber, 1939) Fischback and Terbrüggen (1937a) reported that 20 mgm of vitamin C per day did not prevent thyroid hypertrophy or loss of weight in animals injected with thyrotropic hormone Also, the effect of thyrotropic hormone on tadpole metamorphosis was not lessened by vitamin C (Schäffer, 1936)

7 Clinical studies on vitamin C and hyperthyroidism According to Löhr (1936) vitamin C will reduce the blood iodine in normal and hyperthyroid patients He reports clinical improvement in Graves' disease, but often without the B M R. being altered In 1938 Eitel observed a latent vitamin C deficiency in patients with hyperthyroidism He concluded that the administration of vitamin C to hyperthyroid patients had a symptomatic effect but not a direct action on thyroid activity At the same time Thaddea and Runne (1938) showed

that hyperthyroid patients had a subnormal excretion of vitamin C after a test dose was administered, while two patients with myxedema excreted large quantities of vitamin C   This was confirmed by Lewis (1938) who found that the vitamin C excretion of 5 hyperthyroid patients receiving a constant diet was below normal, but rose to normal in 4 of the 5 cases after thyroidectomy

Summary   Vitamin C deficiency produces hemorrhagic infiltration and hyperplasia of the thyroid gland of scorbutic guinea pigs, the effect being more marked in chronic than acute scurvy   Experimental hyperthyroidism increases the requirements for vitamin C   The effect of vitamin C on the liver glycogen of hyperthyroid animals is not definite, but vitamin C will reduce the creatinuria found in hyperthyroid animals   Hyperthyroidism will also reduce the amount of tissue vitamin C   Vitamin C will partially reduce the B M R of hyperthyroid animals, but its effect on thyroid stimulation produced by the thyrotropic hormone is still controversial   Clinical studies have also shown an increased requirement for vitamin C in hyperthyroidism

THYROID GLAND AND VITAMIN D   *1 Effect of vitamin D deficiency on the thyroid gland*   In early studies on rickets thyroid hypertrophy and hyperplasia was observed (Mellanby and Mellanby, 1921a, Murray, 1923)   Rats maintained on the Steenbock rachitogenic diet also show thyroid hypertrophy and hyperplasia, but the changes produced developed in the presence or absence of vitamin D (Clausen, 1928, Krause and Monroe, 1930, Thompson, 1932, Levine, Remington and v Kolnitz, 1933)   The latter authors definitely showed that the changes produced in the thyroid by Steenbock's rachitogenic diet were due to a deficiency of iodine   The administration of cod liver oil to such rats may supply the daily requirement of iodine   In a later report Remington and Levine (1936) found no effect of the presence or absence of vitamin D on the degree of goiter produced in rats   Drennan, Malcolm and Cox (1931) and Sure (1938) did not observe any effect of vitamin D deficiency on thyroid size   Changes in the thyroid gland reported by other authors (Nitschke, 1933, Bennholdt-Thompson and Wellman, 1934, Uotila, 1938) can be regarded as due to imbalanced diet   Similarly, the decrease of iodine in the thyroid gland during vitamin D deficiency is due to a low intake of iodine (Krause and Monroe, 1930, Levine, Remington and v Kolnitz, 1933)

Although human rickets is said to reduce blood iodine (Nitschke and Doering, 1933, Nitschke, 1934) this observation has not been confirmed (Fasold, 1934a, b, Toepfer, 1934a, b)   According to Kunde and Williams (1926) rabbits thyroidectomized soon after birth developed changes in the bones resembling rickets

2 Effect of vitamin D administration on the thyroid gland   Large doses of irradiated ergosterol were found to increase the B M R (Reed, Thacker, Dillman and Welch, 1933, Nitschke, 1933, Reed, 1934)   Elmer, Geidosz and Scheps (1935) did not observe any effect of vitamin D on the thyroid of guinea pigs   However, Lands and Stoland (1935) observed that while non toxic amounts of irradiated ergosterol had no effect on the thyroid of cats, larger doses increased the amount of colloid   Similar results were obtained by Nitzesco and Bratiano (1936), Bruman and Bromberg (1936), Kucera and Soos (1933) and Goormagh-

tigh and Handovsky (1935) Handovsky and Goormaghtigh (1937) reported that 40 to 60 gamma of vitamin D_2 will produce some increase in thyroid function in dogs, and that with 110 to 230 gamma per day the thyroid was histologically hyperplastic Thyroparathyroidectomized dogs tolerated 600 gamma per day very well, whereas thyroidectomized dogs tolerated this dose very badly with severe damage to the aorta and large arteries They concluded that the action of the thyroid tends to protect the aorta and large arteries, while the action of the parathyroid gland intensifies the injurious action of excessive vitamin D

As parathyroid extract did not increase the B M R. of dogs, Steck, Reed and Miller (1934) decided that vitamin D did not produce its effect by this means Later Deutsch, Reed and Struck (1936) administered vitamin D to 2 thyroparathyroidectomized dogs and did not obtain any increase in metabolic rate as they had in normal dogs Thus the effect of large doses of vitamin D on the metabolic rate depends on an intact thyroid gland Unpublished results have shown that the calorigenic effect of vitamin D is mediated through the thyrotropic hormone of the anterior pituitary gland (cf Reed, Struck and Steck, 1939)

Vitamin D has also been reported to stimulate the oxygen consumption of frog muscle (Gelfan, 1935) Elmer Giedosz and Scheps (1935) found no effect of vitamin D on thyrotropic stimulation of the thyroid gland, and vitamin D did not produce involution of the thyroid of rabbits fed a cabbage diet (Spencer, 1934)

3 Experimental hyperthyroidism and vitamin D It has been shown that the calcium balance during hyperthyroidism may become negative due to a large increase in fecal calcium The administration of vitamin D to normal animals will decrease fecal calcium and simultaneously increase urinary calcium excretion, suggesting that the effect of vitamin D on calcium metabolism during hyperthyroidism should be studied Thus Taylor and Weld (1932) reported that vitamin D decreased the fecal calcium output of hyperthyroid dogs and Pugsley and Anderson (1934a) obtained similar results on rats although a positive calcium balance was not produced It was then demonstrated that, with the proper dosage, vitamin D would produce a calcium balance in thyroid fed rats (Pugsley and Anderson, 1934b)

4 Clinical studies on vitamin D and hyperthyroidism Tibbets, McLean and Aub (1932) found no effect of vitamin D on calcium metabolism during hyperthyroidism, whereas Hansman and Wilson (1934) reported a beneficial effect of vitamin D on calcium balance

Summary A deficiency of vitamin D has no effect upon the thyroid gland The administration of vitamin D to normal animals increases the B M R, probably through its effect on the thyroid gland When given in experimental hyperthyroidism, vitamin D will counteract the increased calcium excretion, returning the calcium balance to normal Further work is needed before the effect of vitamin D on calcium balance in Graves' disease can be ascertained

THYROID GLAND AND VITAMIN E *1 Effect of vitamin E deficiency on the*

thyroid gland   The reports of Paal and Kleine (1933), Singer (1936), Barrie (1937) and Bromskov and Schneider (1939) indicate that vitamin E deficiency in rats produces a picture of decreased thyroid activity   However Telford, Emerson and Evans (1938) did not find any evidence of thyroid hypoplasia in vitamin E deficient female rats, and Biddulph and Meyer (1941, 1942) reported an increase in thyroid activity during vitamin E deficiency   The latter authors found that vitamin E deficiency increased the thyroid weight and B M R of male rats, producing no change in female rats   They also found that the E deficient animals were receiving only about one-fourth as much iodine as control rats on a stock diet   By the addition of iodine to the diet of E deficient males they were able to prevent the changes in the thyroid and the increase in B M R from occurring   They conclude that the effect of vitamin E deficiency in increasing thyroid weight and the B M R is due to a relative deficiency of iodine

Bromskov and Schneider (1939) noted that the thyroid of vitamin E deficient rats resembled that of ovariectomized animals, and that the administration of follicular hormone restored the thyroid function of vitamin E deficient rats Vitamin E would also restore the thyroid of the deficient animals to normal, but had no effect on the thyroid of deficient animals that were ovariectomized   They believe, therefore, that the effect of vitamin E on the thyroid is mediated through the ovary

The administration of vitamin E had no effect on the thyroid of normal rats (Bromskov and Schneider, 1939) or guinea pigs (Giedosz, 1938c), although Paal and Kleine (1933) reported stimulation

2 Thyrotropic hormone and vitamin E   Bromskov and Schneider (1939) reported that the administration of thyrotropic hormone would restore the thyroid of their vitamin E deficient animals to normal   They also found no reduction in the thyrotropic content of the pituitary of E deficient animals, and no change in the antithyrotropic function of the blood of such rats

Summary   Further experiments are necessary to ascertain if the changes reported to occur in the thyroid gland during vitamin E deficiency are a result of this deficiency or are due to other dietary factors

GENERAL SUMMARY

Certain general statements on the interrelations between thyroid function and vitamin metabolism may be made   While some vitamin deficiencies will affect the thyroid gland, a number of the reports emphasize the necessity of controlling such factors as the length of time a deficient diet is fed, the influence of sex difference and whether or not the deficiency is acute or chronic   Each of these variables will influence the results obtained   Also, if a given vitamin deficiency will produce a picture of thyroid hypo- or hyperfunction, it does not follow that overdoses of the same vitamin will produce the opposite effect   Further work is also needed to ascertain the relation of the pituitary gland to the changes produced in the thyroid gland by a vitamin deficiency or the administration of a vitamin

It has been demonstrated that the requirements for vitamins A, B and C are

increased during hyperthyroidism, and if these increased requirements are not met a relative deficiency of the vitamins will ensue   Such a vitamin deficiency may produce changes previously ascribed to an effect of hyperthyroidism per se   Whereas the administration of the necessary vitamins will correct these changes, it should not imply an antithyrogenic action, unless such an effect is demonstrated by further experiments   Conversely, if a vitamin will antagonize certain effects of thyroid feeding, it does not follow that a deficiency of the vitamin alone will increase thyroid activity   In studying vitamin metabolism during experimental hyperthyroidism there is also a sex difference in response, at least in the rat, and a difference in the degree of hyperthyroidism produced by the commonly used doses of thyroxin or thyroid gland   Also, growing rats may gain weight for a considerable period on the same dose of thyroxin or thyroid gland that will produce a progressive loss of weight in adult rats   These factors are of importance in studying the effect of a given vitamin on hyperthyroid animals

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